Coagulation factor II thrombin receptor like 2 (F2RL2) is differentially expressed in brain metastatic breast cancer.

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Breast cancer patients diagnosed with metastasis to the brain¹⁻³ are presented limited treatment options^{4,5}. We mined published microarray data^{6,7} to discover genes associated with brain metastasis in human breast cancer, and identified differential expression of the gene encoding coagulation factor II thrombin receptor like 2, F2RL2, when comparing brain metastatic tissues to primary tumors of the breast. F2RL2 was expressed at significantly lower levels in metastasis to the brain as compared to primary tumors. Coagulation factor II thrombin receptor like 2, its functions and their down-regulation may be relevant to pathways underlying metastasis to the brain in human breast cancer.

Keywords: breast cancer, F2RL2, brain metastasis, central nervous system metastasis, systems biology of breast cancer, targeted therapeutics in breast cancer.

A study of patients treated with treated with trastuzumab, a monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2), reported a rate of metastasis to the central nervous system of 34%². The severity of disease and limited treatment options demand an enhanced understanding of the fundamental transcriptional nature of metastasis to the brain in patients with breast cancer. We mined published microarray data^{6,7} generated using the primary tumors and brain metastatic tissues of patients with human breast cancer to identify genes whose expression was most quantitatively different in brain metastatic tissues. These analyses revealed significant differential expression of F2RL2 in metastases to the brain in patients with human breast cancer.

Methods

We used datasets GSE125989⁷⁶ and GSE43837⁷⁷ and for this differential gene expression analysis of brain metastasis in human 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 F2RL2 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 performed comparative transcriptome analysis of primary and metastatic tumor tissues from patients with breast cancer using published microarray data^{6,7} to describe the transcriptional landscape of brain metastasis in human breast cancer and for the identification of potential therapeutic targets.

F2RL2 is differentially expressed in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

We identified the gene encoding coagulation factor II thrombin receptor like 2, F2RL2, as among the genes whose expression was most significantly different in the brain metastatic tissues of patients with breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in brain metastases based on significance of change in expression as compared to primary tumors of the breast, F2RL2 ranked 56 out of 22277 total transcripts (Table 1), equating to 99.7% differential expression. Differential expression of F2RL2 in metastases to the brain was statistically significant (Table 1; $p=9.88E-05$).

We mined a second microarray dataset to validate differential expression of F2RL2 in brain metastatic breast cancer. We again identified F2RL2 as among the genes whose expression was most different in the brain metastatic tissues of patients with breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in brain metastases based on significance of change in expression as compared to primary tumors of the breast, in this dataset, F2RL2 ranked 2095 out of 61359 total transcripts (Table 2), equating to 96.6% differential expression. Differential expression of F2RL2 in metastases to the brain was again statistically significant (Table 2; $p=0.01302837$).

F2RL2 is expressed at significantly lower levels in metastases to the brain as compared to primary tumors of the breast.

We obtained exact mRNA expression values for F2RL2 from primary tumors of the breast and from brain metastases of patients with breast cancer to evaluate magnitude and direction of change in F2RL2 mRNA expression in metastasis to the brain. F2RL2 was expressed at lower levels in metastasis to the brain as compared to primary tumors of the breast (Figure 1), and this difference was statistically significant (Figure 1; $p < 0.0001$). We calculated a mean fold change of 0.73 ± 0.17 in F2RL2 mRNA expression in brain metastases relative to primary tumors of the breast (Table 1).

Thus, through analysis of published microarray datasets^{6,7}, we found that F2RL2 was among the genes whose expression was most significantly different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast, and that F2RL2 expression was significantly lower in brain metastatic tissues as compared to primary tumors of the breast.

Discussion

We provided evidence here that coagulation factor II thrombin receptor like 2, F2RL2, was among the genes most differentially expressed in metastases to the brain as compared to primary tumors of the breast. Coagulation factor II thrombin receptor like 2 was expressed at significantly lower levels in brain metastatic tissues as compared to primary tumors of the breast. Molecular functions of coagulation factor II thrombin receptor like 2 may be of relevance to the processes which control metastasis of tumor cells from the breast to the brain.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
56	206795_at	9.88E-05	-4.4004504	1.3029	0.73 ± 0.17	F2RL2	coagulation factor II thrombin receptor like 2

Table 1: Differential expression of F2RL2 in brain metastatic tissues as compared to primary tumors in patients with 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 F2RL2 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
2095	g4758325_3p_x_at	0.01302837	-2.5910279	-2.86444	F2RL2	coagulation factor II thrombin receptor like 2

Table 2: Differential expression of F2RL2 in brain metastatic tissues as compared to primary tumors in patients with 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: F2RL2 is expressed at lower levels in brain metastases as compared to primary tumors of the breast in metastatic breast cancer.

The mRNA expression level of F2RL2 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 F2RL2 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.