Cornifelin is differentially expressed in brain metastatic breast cancer.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data^{4,5} to discover genes associated with brain metastasis in breast cancer. We identified significant differential expression of cornifelin, encoded by CNFN, in the brain metastases of patients with metastatic breast cancer. Increased expression of CNFN observed in brain metastatic tissues may be pertinent to understanding the biology underlying colonization of the brain with metastatic breast cancer clones.

Keyword: breast cancer, metastasis, brain metastasis, central nervous system metastasis, CNFN, cornifelin, systems biology of breast cancer, targeted therapeutics in breast cancer.

One study has reported a 34% incidence of central nervous system metastases in patients with breast cancer treated with the monoclonal antibody trastuzumab (Herceptin)². Treatment options for patients with brain metastatic breast cancer are limited^{6,7}. We mined published microarray datasets^{4,5} to understand in an unbiased and systems-level fashion genes whose expression most significantly distinguished brain metastases from primary tumors of the breast, the cells from which these metastatic clones are presumably derived. We found significant differential expression of CNFN in the brain metastases of patients with brain metastatic breast cancer.

Methods

We utilized microarray datasets GSE10893⁴ and GSE100534⁵ for this differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE10893⁴ was generated using Agilent-011521 Human 1A Microarray G4110A technology with $n=11$ primary breast tumors and $n=4$ brain metastases from patients with breast cancer; analysis performed using platform GPL885. GSE100534⁵ was generated using Affymetrix Human Gene 1.0 ST Array technology with $n=16$ primary tumors from patients with breast cancer and $n=3$ brain metastases from patients with brain metastatic breast cancer. 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 CNFN 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 mined published microarray data^{4,5} to discover genes associated with brain metastasis in metastatic breast cancer.

CNFN is differentially expressed in brain metastatic breast cancer.

We identified the cornifelin, encoded by CNFN, as among the genes whose expression changes most significantly when comparing brain metastases to primary tumors of the breast in patients with brain metastatic breast cancer² (Table 1). When sorting each of the genes expressed in brain metastases based on significance of change as compared to primary tumors of the breast, CNFN ranked 153 out of 17418 total transcripts (Table 1), equating to 99.1% differential expression. Differential expression of CNFN in brain metastases in brain metastatic breast cancer was statistically significant (Table 1; $p=3.45E-04$).

We queried a second microarray dataset³ to validate transcriptome-wide differential expression of CNFN in brain metastatic breast cancer. We again found that CNFN was among the genes whose expression changed most significantly when comparing metastases of the brain to primary tumors of the breast in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain metastases based on significance of change as compared to primary tumors of the breast, CNFN ranked 5676 out of 33297 total transcripts (Table 2), equating to 83.0% differential expression. Differential expression of CNFN in brain metastases in brain metastatic breast cancer was statistically significant (Table 2; $p=2.23E-02$).

CNFN is expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast in brain metastatic breast cancer.

We obtained exact mRNA expression values for CNFN from brain metastases and primary tumors of the breast to evaluate direction and statistical significance of change in CNFN expression in brain metastases in patients with breast cancer. CNFN was expressed at higher levels in brain metastases in patients with breast cancer and this difference was statistically significant (Figure 1; $p=0.0123$).

Thus, we found through analysis of published microarray datasets^{4,5} that CNFN was among the genes, globally, whose expression was most different when comparing primary tumors of the breast to brain metastases in patients with breast cancer, and present at significantly higher quantities at the mRNA level in brain metastatic tissues as compared to primary tumors of the breast.

Discussion

We provided evidence here that the gene encoding cornifelin, CNFN, was among those whose expression was most quantitatively different in metastases to the brain when compared to primary tumors of the breast in patients with metastatic breast cancer. Cornifelin is a constituent of the cornified envelope, associates with loricrin and involucrin, and its expression is enriched in the skin of patients with psoriasis⁸. CNFN may be of relevance to the biology underlying any number of steps important for metastasis to the brain: exit from the breast, traversing the vasculature and the blood-brain barrier, and colonization of the brain.

References

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Rank	ID	p-value	t	B	Gene	Gene name
153	7204	3.45E-04	-4.54	0.387618	CNFN	cornifelin

Table 1: CNFN is differentially expressed in brain 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 CNFN 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
5676	8037179	2.23E-02	2.4768153	-3.739334	CNFN	cornifelin

Table 2: CNFN is differentially expressed in brain 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: CNFN is expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast.

The mRNA expression level of CNFN 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 CNFN 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.