The scavenger receptor class A member 5, SCARA5, is differentially expressed in the brain metastases of patients with 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 compare primary and metastatic tumor transcriptomes to discover genes associated with brain metastasis in patients with metastatic breast cancer. We found that the scavenger receptor class A member 5, encoded by SCARA5, was among the genes whose expression was most different in the brain and metastases of patients with metastatic breast cancer as compared to benign or transformed breast tissues. SCARA5 mRNA was present at significantly reduced quantities in brain metastatic tissues as compared to normal breast tissues. Down-regulation of SCARA5 may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer, and irrespective of its importance, appears to be a transcriptional event generally associated with metastasis to the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, SCARA5, scavenger receptor class A member 5, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in trastuzumab-treated patients with breast cancer². This alarmingly high frequency of CNS metastasis events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets, whether they are treatment related or not. We performed a global comparative analysis of primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and decreased expression of the Scavenger receptor class A member 5, encoded by SCARA5, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE52604⁴ and GSE10893⁵ and for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis performed using platform GPL6480. GSE10893, a microarray dataset, was generated using Agilent-011521 Human 1A Microarray G4110A technology with $n=11$ primary tumors of the breast and $n=5$ brain metastases from patients with metastatic breast cancer; platform GPL885 was used for this analysis. 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 SCARA5 gene expression was significantly between benign or malignant breast tissues 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 global comparative transcriptome analysis of metastatic tumor tissues of patients with metastatic breast cancer using published microarray data^{4,5} to describe the transcriptional landscape of brain and lymph node metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

SCARA5 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Identification of differentially expressed genes across datasets in cancer can be difficult and more challenging than when doing so in benign organ tissue and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked whether we could validate differential expression of SCARA5 when comparing brain metastases from patients with breast cancer to normal breast tissue as opposed to primary tumors of the breast, comparing global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁵. Using this strategy, we identified the scavenger receptor class A member 5, SCARA5, as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁴ (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and normal breast tissues, SCARA5 ranked 51 out of 41093 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of SCARA5 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 1; $p=6.93E-14$).

1 Next, we queried a second microarray dataset generated using brain metastases and transformed
2 rather than normal breast tissues from patients with breast cancer to determine whether differential
3 expression of SCARA5 could be observed when comparing primary and metastatic tumors from patients
4 with breast cancer. When sorting each of the genes expressed in brain metastases based on significance of
5 difference as compared to primary tumors of the breast in patients with breast cancer, SCARA5 ranked
6 2661 out of 22575 total transcripts (Table 2), equating to 88.2% differential expression. Differential
7 expression of SCARA5 in the brain metastases of patients with metastatic breast cancer was statistically
8 significant (Table 2; $p=0.03618759$).

9 **SCARA5 is expressed at significantly lower levels in the brain metastases of patients with brain** 10 **metastatic breast cancer.**

11 We obtained exact mRNA expression levels for SCARA5, in normal breast tissues and in brain
12 metastasis of patients with brain metastatic breast cancer to determine direction and statistical
13 significance of change in SCARA5 expression in brain metastatic tissues. SCARA5 was expressed at
14 lower levels in the brain metastases of patients with breast cancer as compared to normal breast tissues,
15 and this difference was statistically significant (Figure 1; $p<0.0001$).

16 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
17 SCARA5 as among the genes whose expression was most different in the brain metastases of patients
18 with breast cancer both when compared to the normal breast; SCARA5 expression, as compared to the
19 benign breast, was significantly lower levels in brain metastases.

20 **Discussion**

21 We provided evidence here that the scavenger receptor class A member 5, encoded by SCARA5,
22 is among the genes whose expression is most different in the brain metastases of patients with brain
23 metastatic brain cancer, and that SCARA5 is expressed at significantly lower levels in brain metastases as
24 compared normal breast tissues. Thus, a ferritin receptor that functions in transport of iron⁶ is among the
25 genes whose expression is most significantly different between the brain metastases Independent
26 evaluation of the effects of ectopic expression of SCARA5 in mouse models of metastatic breast cancer
27 on metastasis to the central nervous system are merited.
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Rank	ID	p-value	t	B	Gene	Gene name
51	A_23_P94103	6.93E-14	-10.4836787	21.412273	SCARA5	scavenger receptor class A member 5

Table 1: SCARA5 is differentially expressed in brain metastases in patients with metastatic breast cancer when compared to normal breast tissues.

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.

Rank	ID	p-value	t	B	Gene	Gene name
2661	16039	0.03618759	-2.2652369	-3.73655	SCARA5	scavenger receptor class A, member 5 (putative)

Table 2: SCARA5 is differentially expressed in brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

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: SCARA5 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to normal breast tissues.

The mRNA expression level of SCARA5 in normal breast tissues (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean SCARA5 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal tissues of the breast, a *p*-value, listed above.