Chemokine dysregulation in brain metastatic breast cancer.

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Metastasis to the central nervous system, including to the brain, is a serious complication of human breast cancer¹. Anti-tumor immunity is a key aspect of control of tumor growth in the human body², and soluble factors including chemokines are important mediators of signaling in the immune system, guiding trafficking of immune cells to anatomical sites of active immune defense³. Through unbiased global gene expression analysis using published microarray datasets⁴⁻⁷, we found that the chemokines CXCL12, CXCL14, CCL13 and CCL19 were among the genes whose expression was most different transcriptome-wide when comparing brain metastases and primary tumors of the breast from patients with human breast cancer. Interestingly, each of these chemokines was present at significantly lower quantities in brain metastatic tissues. These data suggest suppression of chemokine signals to subvert immune surveillance or some other immune function mediated by the directed chemotaxis of immune cells may be important for metastasis to the brain in human breast cancer.

Keywords: anti-tumor immunity, breast cancer, chemokine, CXCL12, CXCL14, CCL13, CCL19, systems biology of breast cancer, targeted therapeutics in breast cancer.

Breast cancer affects women in relatively high numbers⁸, and metastases to the brain affects women diagnosed with breast cancer at relatively high frequency⁹. One report described a 34% incidence of central nervous system metastases in patients with breast cancer treated with trastuzumab¹⁰. To facilitate enhanced understanding of the basic transcriptional biology of brain metastases and to support identification of novel therapeutic targets, we performed global comparative transcriptome profiling using published microarray data⁴⁻⁷ from the primary tumors and brain metastases of women diagnosed with breast cancer. We identified significantly perturbed expression of four chemokines in the brain metastases of patients with breast cancer: CXCL12, CXCL14, CCL13, and CCL19. Each of these chemokines is described to possess important functions in the immune system.

Methods

We used datasets GSE125989⁴, GSE43837⁵, GSE26338⁶, and GSE100534⁷ 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. 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. GSE100534 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 GAS1 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 datasets⁴⁻⁷ to discover genes associated with metastasis to the brain in human metastatic breast cancer.

CXCL12 is differentially expressed in the brain metastases of patients with breast cancer.

We identified the chemokine CXCL12 as among the genes whose expression was most significantly different when comparing brain metastatic tissues and primary tumors of the breast in human breast cancer. 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, CXCL12 ranked 20 out of 22277 total transcripts (Table 1). Differential expression of CXCL12 in the brain metastases of patients with breast cancer was statistically significant (Table 1; $p=9E-06$).

We queried a second microarray dataset to attempt to validate transcriptome-wide differential expression of CXCL12 in brain metastatic breast cancer. When sorting each of the genes expressed in

1 brain metastases based on significance of change in expression as compared to primary tumors of the
2 breast, in this second dataset, CXCL12 ranked 1257 out of 22575 total transcripts (Table 2). Differential
3 expression of CXCL12 in the brain metastases of patients with breast cancer was again statistically
4 significant (Table 2; $p=1.05E-02$).

5 **CXCL14 is differentially expressed in the brain metastases of patients with breast cancer.**

6 We found a second chemokine, CXCL14, among the genes whose expression was most
7 significantly different when comparing brain metastatic tissues and primary tumors of the breast in human
8 breast cancer. When sorting each of the genes expressed in brain metastases based on significance of
9 change in expression as compared to primary tumors of the breast, CXCL14 ranked 188 out of 22277
10 total transcripts (Table 3). Differential expression of CXCL14 in the brain metastases of patients with
11 breast cancer was statistically significant (Table 3; $p=1.32E-03$).

12 We queried a second microarray dataset to attempt to validate transcriptome-wide differential
13 expression of CXCL14 in brain metastatic breast cancer. When sorting each of the genes expressed in
14 brain metastases based on significance of change in expression as compared to primary tumors of the
15 breast, in this second dataset, CXCL14 ranked 5144 out of 33297 total transcripts (Table 4). Differential
16 expression of CXCL14 in the brain metastases of patients with breast cancer was again statistically
17 significant (Table 4; $p=1.74E-02$).

18 **CCL13 is differentially expressed in the brain metastases of patients with breast cancer.**

19 Our systems-level analysis identified a third chemokine, CCL13, among the genes whose
20 expression was most significantly different when comparing brain metastatic tissues and primary tumors
21 of the breast in human breast cancer. When sorting each of the genes expressed in brain metastases based
22 on significance of change in expression as compared to primary tumors of the breast, CCL13 ranked 18
23 out of 61359 total transcripts (Table 5). Differential expression of CCL13 in the brain metastases of
24 patients with breast cancer was statistically significant (Table 5; $p=0.0000658$).

25 We queried a second microarray dataset to attempt to validate transcriptome-wide differential
26 expression of CCL13 in brain metastatic breast cancer. When sorting each of the genes expressed in
27 brain metastases based on significance of change in expression as compared to primary tumors of the
28 breast, in this second dataset, CCL13 ranked 2552 out of 22575 total transcripts (Table 6). Differential
expression of CCL13 in the brain metastases of patients with breast cancer was again statistically
significant (Table 6; $p=3.23E-02$).

29 **CCL19 is differentially expressed in the brain metastases of patients with breast cancer.**

30 We identified a fourth chemokine, CCL19, among the genes whose expression was most
31 significantly different when comparing brain metastatic tissues and primary tumors of the breast in human
32 breast cancer. When sorting each of the genes expressed in brain metastases based on significance of
33 change in expression as compared to primary tumors of the breast, CCL19 ranked 47 out of 22277 total
34 transcripts (Table 7). Differential expression of CCL19 in the brain metastases of patients with breast
35 cancer was statistically significant (Table 7; $p=6.04E-05$).

We queried a second microarray dataset to attempt to validate transcriptome-wide differential expression of CCL13 in brain metastatic breast cancer. 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 second dataset, CCL13 ranked 3119 out of 22575 total transcripts (Table 8). Differential expression of CCL13 in the brain metastases of patients with breast cancer was again statistically significant (Table 8; $p=4.46E-02$).

CXCL12 is expressed at significantly lower levels in brain metastatic tissues as compared to primary tumors of the breast in patients with breast cancer.

We obtained exact mRNA expression levels for CXCL12 from primary tumors of the breast and from brain metastases from humans with breast cancer to evaluate magnitude and direction of change in CXCL12 gene expression in brain metastatic breast cancer. CXCL12 was expressed at lower levels in brain metastatic tissues as compared to primary tumors of the breast; decreased expression of CXCL12 in brain metastases was statistically significant (Figure 1; $p=0.0019$). We calculated a mean fold change of 0.814 ± 0.196 in CXCL12 gene expression when comparing brain metastases to primary tumors of the breast (Table 1).

CXCL14 is expressed at significantly lower levels in brain metastatic tissues as compared to primary tumors of the breast in patients with breast cancer.

We also obtained exact mRNA expression levels for CXCL14 from primary tumors of the breast and from brain metastases from humans with breast cancer to evaluate magnitude and direction of change in CXCL14 gene expression in brain metastatic breast cancer. CXCL14 was expressed at lower levels in brain metastatic tissues as compared to primary tumors of the breast; decreased expression of CXCL14 in brain metastases was statistically significant (Figure 3; $p=0.0027$). We calculated a mean fold change of 0.814 ± 0.210 in CXCL14 gene expression when comparing brain metastases to primary tumors of the breast (Table 3).

CCL13 is expressed at significantly lower levels in brain metastatic tissues as compared to primary tumors of the breast in patients with breast cancer.

Next, we obtained exact mRNA expression levels for CCL13 from primary tumors of the breast and from brain metastases from humans with breast cancer to evaluate magnitude and direction of change in CCL13 gene expression in brain metastatic breast cancer. CCL13 was expressed at lower levels in brain metastatic tissues as compared to primary tumors of the breast; decreased expression of CCL13 in brain metastases was statistically significant (Figure 4; $p=0.0043$). We calculated a mean fold change of 0.296 ± 0.253 in CCL13 gene expression when comparing brain metastases to primary tumors of the breast (Table 5).

CCL19 is expressed at significantly lower levels in brain metastatic tissues as compared to primary tumors of the breast in patients with breast cancer.

Next, we obtained exact mRNA expression levels for CCL19 from primary tumors of the breast and from brain metastases from humans with breast cancer to evaluate magnitude and direction of change in CCL19 gene expression in brain metastatic breast cancer. CCL19 was expressed at lower levels in brain metastatic tissues as compared to primary tumors of the breast; decreased expression of CCL19 in

1 brain metastases was statistically significant (Figure 4; $p=0.0001$). We calculated a mean fold change of
2 0.709 ± 0.161 in CCL19 gene expression when comparing brain metastases to primary tumors of the
3 breast (Table 7).

4 Together, using blind comparative transcriptome analysis, we discovered concurrent
5 dysregulation of multiple chemokines - CXCL12, CXCL14, CCL13 and CCL19 - in the brain metastases
6 and of patients with human breast cancer. Each of these chemokines was present at significantly reduced
7 quantities in brain metastases relative to primary breast tumors in patients with breast cancer.

8 **Discussion**

9 CXCL12, CXCL14, CCL13 and CCL19 together have critical functions in diverse biological
10 processes relevant to tumor maintenance and metastasis including chemotaxis of immature dendritic cells
11 and monocytes as well as activation of eosinophil respiratory burst¹¹⁻¹⁷. In particular, CCL19, also known
12 as MIP-3 β /ELC, functions in recruitment and mobilization of dendritic cells to the lymph nodes¹⁷, and
13 CXCL14 (BRAK) functions as an inhibitor of angiogenesis¹³. These data suggest multifactorial
14 contributions of chemokines to brain metastasis in human breast cancer, including failure of immune
15 surveillance in the lymph nodes resulting from decreased CCL19 in conjunction with loss of normal
16 tumor growth control mechanisms: failure to restrict angiogenesis due to decreased CXCL14. Molecular
17 mechanisms by which chemokines exert their function in human metastatic breast cancer will have to
18 experimentally evaluated but we found here the transcriptome-wide differential expression of multiple
19 chemokines in brain metastatic breast cancer.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
20	209687_at	9E-06	-5.2029255	3.4165	0.814 ± 0.196	CXCL12	C-X-C motif chemokine ligand 12

Table 1: CXCL12 is differentially expressed in brain metastases in human 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 CXCL12 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
1257	20858	1.05E-02	-2.93	-2.75169	CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)

Table 2: CXCL12 is differentially expressed in brain metastases in human 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.

Rank	ID	p-value	t	B	FC	Gene	Gene name
188	218002_s_at	1.32E-03	-3.4960027	-0.9776	0.814 ± 0.210	CXCL14	C-X-C motif chemokine ligand 14

Table 3: CXCL14 is differentially expressed in brain metastases in human 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 CXCL14 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
5144	8114249	1.74E-02	-2.591706	-3.51485	CXCL14	C-X-C motif chemokine ligand 14

Table 4: CXCL14 is differentially expressed in brain metastases in human 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.

Rank	ID	p-value	t	B	FC	Gene	Gene name
18	Hs.11383.1.A1_3p_at	0.0000658	-4.4220702	1.34035	0.296 ± 0.253	CCL13	C-C motif chemokine ligand 13

Table 5: CCL13 is differentially expressed in brain metastases in human 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 CCL13 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
2552	3553	3.23E-02	-2.36	-3.76768	CCL13	chemokine (C-C motif) ligand 13

Table 6: CCL13 is differentially expressed in brain metastases in human 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.

Rank	ID	p-value	t	B	FC	Gene	Gene name
47	210072_at	6.04E-05	-4.5669228	1.7374	0.709 ± 0.161	CCL19	C-C motif chemokine ligand 19

Table 7: CCL19 is differentially expressed in brain metastases in human 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 CCL19 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
3119	3485	4.46E-02	-2.20	-4.05404	CCL19	chemokine (C-C motif) ligand 19

Table 8: CCL19 is differentially expressed in brain metastases in human 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: CXCL12 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in human metastatic breast cancer.

The mRNA expression level of CXCL12 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 CXCL12 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.

Figure 2: CXCL14 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in human metastatic breast cancer.

The mRNA expression level of CXCL14 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 CXCL14 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.

Figure 3: CCL13 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in human metastatic breast cancer.

The mRNA expression level of CCL13 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 CCL13 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.

Figure 4: CCL19 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in human metastatic breast cancer.

The mRNA expression level of CCL19 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 CCL19 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.