The immunoglobulin heavy chain variable gene segment 4-31, IGHV 4-31, is differentially expressed in the brain and lymph nodes of patients with metastatic breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

One study reported the incidence of central nervous system metastases in breast cancer patients treated with trastuzumab as 34%¹. We mined published microarray data²⁻⁴ to discover genes associated with brain metastasis in breast cancer. We identified significant differential expression of the immunoglobulin heavy chain variable gene segment IGHV 4-31 in the metastases of patients with metastatic breast cancer, both in the lymph nodes and in the brain. We recently described the differential expression of the immunoglobulin light chain kappa constant locus in the brain metastases of patients with metastatic breast cancer⁵. These data suggest both heavy and light chain gene segments may potentially be among the loci whose expression is most significantly altered transcriptome-wide when comparing primary tumors of the breast and brain metastases in patients with metastatic breast cancer. IGHV 4-31 may be relevant to the biology underlying colonization of the brain with metastatic breast cancer clones.

Keyword: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, IGHV 4-31, systems biology of breast cancer, targeted therapeutics in breast cancer.

One study reported a 34% incidence of central nervous system metastases in patients with breast cancer treated with the monoclonal antibody trastuzumab (Herceptin)¹. Treatment options for patients with brain metastatic breast cancer are limited^{6,7}. We mined published microarray datasets²⁻⁴ 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 IGHV 4-31 in the brain metastases of patients with brain metastatic breast cancer.

Methods

We utilized microarray datasets GSE125989², GSE43837³ and GSE124648⁴ for this 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. GSE124648, a microarray dataset, was generated using Affymetrix Human Genome U133A Array technology with $n=10$ normal breast tissues and $n=44$ lymph node metastases from patients with breast cancer; platform GPL96 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 IGHV 4-31 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 data²⁻⁴ to discover genes associated with brain metastasis in breast cancer.

IGHV 4-31 is differentially expressed in brain metastatic breast cancer.

We identified the immunoglobulin heavy chain variable gene segment 4-31, IGHV 4-31, as among the genes most differentially expressed gene transcriptome-wide 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, IGHV 4-31 along with several other IGH gene segments ranked 66 out of 22277 total transcripts (Table 1), equating to 99.7% differential expression. Differential expression of IGHV 4-31 in brain metastases in brain metastatic breast cancer was statistically significant (Table 1; $p=1.35E-04$).

We queried a second microarray dataset³ to validate transcriptome-wide differential expression of IGHV 4-31 in brain metastatic breast cancer. We again found that IGHV 4-31 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, IGHV 4-31 ranked 5451 out of 61359 total transcripts (Table 2), equating to 91.1% differential expression. Differential expression of IGHV 4-31 in brain metastases in brain metastatic breast cancer was statistically significant (Table 2; $p=0.04068513$).

IGHV 4-31 is differentially expressed in lymph nodes of patients with metastatic breast cancer.

We asked whether we could validate differential expression of IGHV 4-31 was specific to brain metastasis or if we could observe differential expression of IGHV 4-31 in lymph node metastasis. Comparison of global gene expression profiles of 10 normal breast tissues to 44 lymph node metastases⁵ revealed that IGHV 4-31 was also among the genes whose expression was most significantly different transcriptome-wide between the breast and lymph node metastases in patients with metastatic breast cancer (Table 3). When sorting each of the genes expressed in lymph node metastases based on significance of difference in expression as compared to normal breast tissues, IGHV 4-31 ranked 3008 out of 22283 total transcripts (Table 3), equating to 86.5% differential expression. Differential expression of IGHV 4-31 in lymph node metastases from patients with metastatic breast cancer was statistically significant (Table 3; $p=5.9E-04$).

IGHV 4-31 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in brain metastatic breast cancer.

We obtained exact mRNA expression values for IGHV 4-31 from brain metastases and primary tumors of the breast to evaluate magnitude and direction of change in IGHV 4-31 expression in brain metastases in patients with breast cancer. IGHV 4-31 was expressed at lower levels in brain metastases in patients with breast cancer and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 0.77 ± 0.17 in IGHV 4-31 expression when comparing brain metastatic tissues to primary tumors of the breast (Table 1).

Thus, we found that IGHV 4-31 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, with significantly decreased expressed of IGHV 4-31 in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast.

Discussion

An enhanced understanding of brain metastases can facilitate development of novel therapeutic strategies. We identified the immunoglobulin heavy chain variable gene segment 4-31, IGHV 4-31, as among the genes most differentially expressed in brain metastases when compared to primary tumors of the breast in patients with breast cancer. We have recently described the differential expression and down-regulation of the immunoglobulin light chain kappa constant gene segment, IGKC⁵, the terminal deoxynucleotidyl transferase TdT⁶, and the joining chain of multimeric IgA and IgM, J chain⁷, in the brain metastases of patients with metastatic breast cancer. If not attributable to tissue contamination with tumor-infiltrating lymphocytes, these data would indicate that a fourth component specific to B-lymphocytes was expressed by primary tumors of the breast and importantly here, significantly down-regulated in the brain metastases of patients with metastatic breast cancer. It remains unclear why we observe silencing of B-lymphocyte-specific gene expression in brain metastatic breast cancer, but one possibility is that tumor-intrinsic humoral responses exist to some extent, provoked by a stimulus of unknown origin, that these tumor-intrinsic humoral responses are prohibitive to migration, invasion or colonization of the brain and that silencing of this tumor-intrinsic humoral gene expression, or limiting the diversity of the humoral responses produced, thus removes one layer of immune privilege which protects the organism from central nervous system dissemination of tumor clones from the breast.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
66	216510_x_at	1.35E-04	-4.2942622	1.0274	0.77 ± 0.17	IGHV3-23 IGHV4-31 IGHM IGHG IGHA1	immunoglobulin heavy variable 3-23 immunoglobulin heavy variable 4-31 immunoglobulin heavy constant mu immunoglobulin heavy constant gamma 1 (G1m marker) immunoglobulin heavy constant alpha 1

Table 1: IGHV 4-31 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 IGHV 4-31 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
5451	Hs.288553.0.S1_3p_s_at	0.04068513	-2.1105619	-3.73486	IGHV4-31	immunoglobulin heavy variable 4-31

Table 2: IGHV 4-31 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.

Rank	ID	p-value	t	B	Gene	Gene name
3008	211641_x_at	5.9E-04	3.64502	-0.9644	IGHV4-31/// IGHM/// IGHG3/// IGHG1/// IGHD/// IGHA2/// IGHA1	immunoglobulin heavy variable 4-31 immunoglobulin heavy constant mu immunoglobulin heavy constant gamma 3 (G3m marker) immunoglobulin heavy constant gamma 1 (G1m marker) immunoglobulin heavy constant delta immunoglobulin heavy constant alpha 2 (A2m marker) immunoglobulin heavy constant alpha 1

Table 3: IGHV 4-31 is differentially expressed in lymph node metastases in 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.

Figure 1: IGHV 4-31 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with brain metastatic breast cancer.

The mRNA expression level of IGHV 4-31 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 IGHV 4-31 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.