The joining chain of multimeric IgA and IgM is differentially expressed in metastatic breast cancer, both in metastases to the brain and to the lymph nodes.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data⁴⁻⁶ to compare primary and metastatic tumor transcriptomes to discover genes associated with brain metastasis in patients with metastatic breast cancer. We found that the joining chain of multimeric IgA and IgM, J chain, encoded by JCHAIN, was among the genes whose expression was most different in the metastatic tumor tissues of patients with metastatic breast cancer, both in metastases to brain and to the lymph nodes when compared to primary tumors of the breast or to normal breast tissues, respectively. We have recently demonstrated transcriptome-wide differential expression and down-regulation of IGKC⁷ and TdT⁸ in brain metastasis of patients with metastatic breast cancer. Molecular functions of JCHAIN⁹⁻¹¹ and down-regulation of these functions may be important for metastasis of primary tumor-derived cancer cells to the lymph nodes and to the brain in humans with metastatic breast cancer, and these data suggest some level of common origin for metastases that reside in the lymph nodes and colonize the brain.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, JCHAIN, joining chain of multimeric IgA and IgM, J chain, 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 metastatic breast cancer^{4,5}. We discovered transcriptome-wide differential expression of JCHAIN in brain and lymph node metastases of patients with metastatic breast cancer.

Methods

We used datasets GSE125989⁴, GSE124648⁵ and GSE52604⁶ 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. 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. 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. 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 JCHAIN 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 global comparative transcriptome analysis of the primary and metastatic tumor tissues of patients with brain and lymph node metastatic breast cancer using published microarray data^{4,5} to describe the transcriptional landscape of metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

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

We identified the joining chain of multimeric IgA and IgM, encoded by JCHAIN, 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 as compared to the primary tumors of patients with brain metastatic breast cancer, JCHAIN ranked 19 out of 22277 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of JCHAIN in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=5.9E-06$).

We asked whether we could validate differential expression of JCHAIN was specific to brain metastasis or if we could observe differential expression of JCHAIN in lymph node metastasis. Comparison of global gene expression profiles of 10 normal breast tissues breast to 44 lymph node metastases⁵ revealed that JCHAIN was among the genes whose expression was most significantly different transcriptome-wide between the breast and lymph metastases in patients with metastatic breast cancer (Table 2). When sorting each of the genes expressed in lymph node metastases based on

significance of difference in expression as compared to normal breast tissues, JCHAIN ranked 912 out of 22283 total transcripts (Table 2), equating to 95.9% differential expression. Differential expression of JCHAIN in lymph node metastases from patients with metastatic breast cancer was statistically significant (Table 2; $p=5.82E-07$).

Finally, interrogation of a third microarray dataset revealed that JCHAIN was again among the genes most differentially expressed in metastatic breast cancer, in brain metastases when compared to normal breast tissues. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to normal breast tissues, JCHAIN ranked 12 out of 41093 total transcripts (Table 3), equating to 99.97% differential expression. Differential expression of JCHAIN in brain metastases from patients with metastatic breast cancer, here when compared to normal tissues of the breast, was statistically significant (Table 3; $p=6.58E-17$).

JCHAIN is expressed at significantly lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for JCHAIN, in primary tumors of the breast and in brain metastasis of patients with metastatic breast cancer to determine magnitude and direction of change in JCHAIN expression in brain metastatic tissues. JCHAIN was expressed at lower levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 0.62 ± 0.25 in JCHAIN expression when comparing primary tumors of the breast to brain metastatic tissues in patients with metastatic breast cancer (Table 1).

We also obtained exact mRNA expression levels for JCHAIN, in normal breast tissues and in brain metastases of patients with metastatic breast cancer to evaluate direction and statistical significance of change in JCHAIN expression in brain metastases relative to the non-transformed breast. JCHAIN was expressed at lower levels in the brain metastases of patients with breast cancer as compared to normal breast tissues, and this difference was statistically significant (Figure 2; $p<0.0001$).

Thus, by mining published microarray data⁴⁻⁶ in an unbiased and systematic fashion, we identified JCHAIN as a differentially expressed gene in metastatic breast cancer, both in brain metastases when compared to primary tumors of the breast as well as in lymph node metastases when compared to normal breast tissues. JCHAIN was expressed at significantly lower levels in brain metastases, both when compared to primary tumors of the breast and to normal breast tissues.

Discussion

We provided evidence here that the joining chain of multimeric IgA and IgM, encoded by JCHAIN is among the genes whose expression is most different in the brain and lymph node metastases of patients with metastatic breast cancer. JCHAIN may be of relevance to any number of steps required for metastasis to the lymph nodes and to the brain, and differential expression of JCHAIN in metastases of the brain and of the lymph nodes suggests some level of common origin for metastases that originate in the breast, reside in the lymph nodes and colonize the brain. It has been reported that the J chain is important for transport of immunoglobulins, binding to the secretory component on the basolateral membrane of secretory epithelial cells⁹⁻¹¹. It remains unclear to why multiple components of immunoglobulin gene expression including the immunoglobulin light chain kappa gene segment, terminal deoxynucleotidyl transferase and J chain are among the most differentially expressed genes when comparing normal breast tissue or primary tumors of the breast to metastases of the brain, but it appears very likely that down-regulation of these components is an integral part of the process by which cells derived from the primary tumor exit the breast, traverse the vasculature, evade detection and elimination by the lymph nodes, cross the blood-brain barrier, migrate to and colonize the brain.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
19	212592_at	5.9E-06	-5.3429845	3.7879	0.62 ± 0.25	JCHAIN	joining chain of multimeric IgA and IgM

Table 1: JCHAIN is differentially expressed in brain metastases in 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, fold change (FC) of JCHAIN 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
912	212592_at	5.82E-07	5.645944	5.711612	JCHAIN	joining chain of multimeric IgA and IgM

Table 2: JCHAIN is differentially expressed in lymph node metastases in 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.

Rank	ID	p-value	t	B	Gene	Gene name
12	6.58E-17	-15.8243855	35.783516	-6.2887286	JCHAIN	joining chain of multimeric IgA and IgM

Table 3: JCHAIN is differentially expressed in brain metastases in metastatic breast cancer when compared to normal breast tissue.

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: JCHAIN is expressed at significantly lower levels in the brain metastases of patients with metastatic breast cancer.

The mRNA expression level of JCHAIN 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 JCHAIN 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: JCHAIN is expressed at significantly lower levels in the brain metastases of patients with metastatic breast cancer.

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