The matrix metalloproteinase 2 (MMP2) is differentially expressed and down-regulated in metastases to the lymph node and the brain in 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 matrix metalloproteinase 2, MMP2 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 normal breast tissue, respectively. We observed significant down-regulation of MMP2 in metastasis to the brain and in metastasis to the lymph nodes. Molecular functions and down-regulation of MMP2 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 combined 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, MMP2, matrix metalloproteinase 2, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in patients treated with trastuzumab for breast cancer². More recently, the NEfERT-T clinical trial which compared administration of either neratinib or trastuzumab in conjunction with paclitaxel demonstrated that in a randomized, controlled setting, in breast cancer patients treated with neratinib, not only was the incidence of central nervous system recurrence significantly lower, the time to central nervous system metastasis was significantly delayed as compared to patients administered trastuzumab. The alarmingly high rate of central nervous system metastasis described, as well as data, both anecdotal² and from a randomized, controlled setting⁶ illustrating that treatment with trastuzumab may be associated with these 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 MMP2 in brain and lymph node metastases of patients with metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE124648⁵ 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. 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 MMP2 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.

MMP2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified matrix metalloproteinase 2, encoded by MMP2, 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, MMP2 ranked 27 out of 22277 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of MMP2 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=1.35E-05$).

We asked whether we could validate differential expression of MMP2 was specific to brain metastasis or if we could observe differential expression of MMP2 in lymph node metastasis. Comparison of global gene expression profiles of 10 normal breast tissues to 44 lymph node metastases⁵

revealed that MMP2 was 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 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, MMP2 ranked 116 out of 22283 total transcripts (Table 2), equating to 99.5% differential expression. Differential expression of MMP2 in lymph node metastases from patients with metastatic breast cancer was statistically significant (Table 2; $p=1.06E-13$).

MMP2 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for MMP2, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine magnitude and direction of change in MMP2 expression in brain metastatic tissues. MMP2 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 0.84 ± 0.09 in MMP2 expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

MMP2 is expressed at significantly lower levels in the lymph node metastases of patients with brain metastatic breast cancer.

We also obtained exact mRNA expression levels for MMP2, in normal breast tissues and in lymph node metastasis of patients with metastatic breast cancer to determine magnitude and direction of change in MMP2 expression in brain metastatic tissues. MMP2 was expressed at lower levels in the lymph node metastases of patients with breast cancer as compared to normal (benign) tissues of the breast, and this difference was statistically significant (Figure 2; $p<0.0001$). We calculated a mean fold change of 0.73 ± 0.09 in MMP2 expression when comparing normal breast tissues to lymph node metastases in patients with breast cancer (Table 2).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified MMP2 as a differentially expressed gene in metastatic breast cancer, both in brain metastases and in lymph node metastases when compared to tissues of the breast, transformed and benign; MMP2 was expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast and in lymph node metastases when compared to benign tissues of the breast.

Discussion

We provided evidence here that matrix metalloproteinase 2, encoded by MMP2 is among the genes whose expression is most different in the brain and lymph node metastases of patients with metastatic breast cancer. While studies have documented increased expression of MMP2 in brain metastasis in rat models of breast cancer^{7,8}, and while the brain metastases of patients with melanoma have been described to over-express MMP2^{9,10}, we found that MMP2 was expressed at significantly lower levels in brain metastases of patients with metastatic breast cancer. Our study utilized microarray data generated using primary and metastatic tissues from humans with breast cancer, not cell lines or experimental models. MMP2 may be of relevance to any number of steps required for metastasis to the lymph nodes and to the brain. Differential expression and down-regulation of MMP2 in metastasis to the lymph nodes and to the brain suggests some level of common origin for metastases that reside in the lymph nodes, successfully evade immune clearance, and subsequently colonize the brain.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
27	201069_at	1.35E-05	-5.0686142	3.0605	0.84 ± 0.09	MMP2	matrix metalloproteinase 2

Table 1: MMP2 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 MMP2 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	FC	Gene	Gene name
116	201069_at	1.06E-13	9.796443	20.97209	0.73 ± 0.09	MMP2	matrix metalloproteinase 2

Table 2: MMP2 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: MMP2 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of MMP2 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 MMP2 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: MMP2 is expressed at significantly lower levels in the lymph node metastases of patients with metastatic breast cancer.

The mRNA expression level of MMP2 in normal tissues of the breast (left) and in the lymph node metastases of metastatic breast cancer patients (right) is graphically represented here with mean MMP2 levels marked and the result of a statistical test evaluating difference in mRNA expression level between lymph node metastases and normal breast tissues, a *p*-value, listed above.