MMP13 is differentially expressed and down-regulated in brain metastatic breast cancer.

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Brain metastases are a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data^{4,5} to discover genes associated with brain metastasis in patients with brain metastatic breast cancer. We found that the gene encoding the matrix metalloproteinase 13, MMP13, was among the genes most differentially expressed in the brain metastases of patients with brain metastatic breast cancer. MMP13 may be of relevance to the biology underlying metastasis to the brain in humans with metastatic breast cancer. MMP13 now joins MMP14, MMP11 and MMP2 among matrix metalloproteinases we have found to be differentially expressed and down-regulated in the brain metastases of humans with metastatic breast cancer⁶⁻⁸.

Keywords: breast cancer, brain metastases, central nervous system metastases, MMP13, 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 metastases events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets. We performed a global comparative analysis of transcription in the brain metastases of patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and down-regulation of the matrix metalloproteinase MMP13 in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE43837⁵ 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. 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 MMP13 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 metastatic breast cancer for the discovery of novel therapeutic targets using published microarray data^{4,5}.

MMP13 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the matrix metalloproteinase MMP13 as among the genes whose expression was most significantly different, transcriptome-wide, 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, MMP13 ranked 84 out of 22277 total transcripts (Table 1), equating to 99.6% differential expression. Differential expression of MMP13 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=2.39E-04$).

We mined a second microarray dataset⁵ to attempt to validate global differential expression of MMP13 in brain metastatic breast cancer. Again, we found that MMP13 was among the genes whose expression changed most in the brain metastases of patients with brain metastatic breast cancer when compared to primary tumors of the breast. 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, MMP13 ranked 143 out of 61359 total transcripts, equating to 99.8% differential expression (Table 2). Differential expression of MMP13 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 2; $p=0.00065789$).

MMP13 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for MMP13, 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 MMP13 expression in brain metastatic tissues. MMP13 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; $p=0.0018$). We calculated a mean fold change of 0.61 ± 0.34 in MMP13 expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

We also obtained exact mRNA expression levels for MMP13, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer from the second microarray dataset analyzed to determine magnitude and direction of change in MMP13 expression in brain metastatic tissues. MMP13 was expressed at lower levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, but in this case the difference was not deemed statistically significant (Figure 2; $p=0.1083$). We calculated a mean fold change of 0.08 ± 0.09 in MMP13 expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 2).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the matrix metalloproteinase MMP13 as among the genes whose expression was most different in the brain metastases of patients with breast cancer; MMP13 was expressed at lower levels in brain metastases as compared to primary tumors of the breast in both datasets analyzed, and in one case, this difference was statistically significant.

Discussion

We provided evidence here that the matrix metalloproteinase MMP13 is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. Multiple lines of investigation we have conducted suggest that as a family, modulating gene expression of the matrix metalloproteinases is an important event during metastatic progression of human breast cancer. We have previously described the differential expression and down-regulation of MMP14 in the brain and bone metastases of humans with metastatic breast cancer⁶. We have recently demonstrated that the matrix metalloproteinase MMP11 is down-regulated and differentially expressed in brain metastatic tissues of patients with metastatic breast cancer, and that bevacizumab treatment phenocopied this change⁷. Finally, we recently discovered that matrix metalloproteinase, MMP2 is also among the genes most differentially expressed in metastatic breast cancer, present at significantly decreased quantity at the mRNA level in the brain and lymph node metastases of humans with metastatic breast cancer⁸. Thus, through blind, comparative transcriptome analyses of published microarray data we have in total demonstrated the differential expression and down-regulation of matrix metalloproteinases MMP2, MMP11, MMP13 and MMP14 in the brain metastases of humans with metastatic breast cancer. The targets of MMP13 in the extracellular matrix and down-regulation of its catalytic activities through modulation of its expression may be of relevance to the biology underlying metastasis to the brain in patients with brain metastatic breast cancer.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
84	205959_at	2.39E-04	-4.0986489	0.5242	0.61 ± 0.34	MMP13	matrix metalloproteinase 13

Table 1: MMP13 is differentially expressed in brain metastases 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 MMP13 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
143	g13027796_3p_at	0.00065789	-3.6745914	-0.5014	0.08 ± 0.09	MMP13	matrix metalloproteinase 13

Table 2: MMP13 is differentially expressed in brain metastases 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.

Figure 1: MMP13 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of MMP13 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 MMP13 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: MMP13 is expressed at lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of MMP13 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 MMP13 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. The Y-axis is presented in natural logarithm (ln).