GRM8 is differentially expressed in the brain metastases of 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 glutamate metabotropic receptor 8, encoded by GRM8, was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to normal breast tissues and primary tumors of the breast. GRM8 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast and to normal breast tissues. Up-regulation of GRM8 expression may contribute to metastasis of tumor cells from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, glutamate metabotropic receptor 8, GRM8, 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 trial⁶ 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 the benign breast, primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and decreased expression of the glutamate metabotropic receptor 8, GRM8, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE52604⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE10893 was generated using Agilent-011521 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. 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 GRM8 gene expression was significantly between primary tumors of the breast 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 metastatic tumor tissues of patients with metastatic breast cancer using published microarray data^{4,5} to describe the transcriptional landscape of brain metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

GRM8 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through analysis of published microarray data⁴, we identified the glutamate metabotropic receptor 8 GRM8 as a differentially expressed gene in the breast metastatic tissues of humans with breast cancer (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, GRM8 ranked 176 out of 22575 total transcripts (Table 1), equating to 99.2% differential expression. Differential expression of GRM8 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=5.37E-04$).

Identification of differentially expressed genes across datasets in cancer can be difficult and more challenging than when doing so in benign organ tissue and sorted cell populations due to higher levels of

heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked how gene expression was most significantly different between brain metastases from patients with breast cancer and normal breast tissue as opposed to primary tumors of the breast, by comparing global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁵. Using this strategy, we validated GRM8 as a differentially expressed gene in the brain metastases of patients with brain metastatic breast cancer⁵ (Table 2). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and normal breast tissues, GRM8 ranked 16 out of 41093 total transcripts (Table 2), equating to 99.96% differential expression. Differential expression of GRM8 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=9.54E-19$).

Thus, differential expression of GRM8 was conserved both when compared to normal tissues of the breast and to primary tumors of the breast in patients with metastatic breast cancer, in two independent microarray datasets^{4,5}.

GRM8 is expressed at higher levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for GRM8, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in GRM8 expression in brain metastatic tissues. GRM8 was expressed at higher 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; $p=0.1211$).

We obtained exact mRNA expression levels for GRM8, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in GRM8 expression in brain metastatic tissues. GRM8 was expressed at higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, but this difference was not statistically significant (Figure 2; $p<0.0001$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the glutamate metabotropic receptor 8, GRM8 as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer both when compared to the normal breast and to primary tumors of the breast; GRM8 expression, as compared to primary tumors of the breast and to normal breast tissues, was higher in metastasis to the brain.

Discussion

We provided evidence here that the glutamate metabotropic receptor 8, encoded by GRM8, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer. Evaluation of the effects of genetic depletion of GRM8 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited, but species-specific differences in cell-type specific interaction partners and regulators, as well as key differences in host immunology between mouse and man will inherently complicate interpretation of data generated using xenograft and autochthonous mouse models.

References

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Rank	ID	p-value	t	B	Gene	Gene name
176	8714	5.37E-04	4.40	0.00165	GRM8	glutamate receptor, metabotropic 8

Table 1: GRM8 is differentially expressed in brain metastases of patients with 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
16	A_32_P8221	9.54E-19	14.3179179	32.117739	GRM8	glutamate metabotropic receptor 8

Table 2: GRM8 is differentially expressed in brain metastases in patients with 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: GRM8 is expressed at higher levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

The mRNA expression level of GRM8 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 GRM8 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: GRM8 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer as compared to normal breast tissues.

The mRNA expression level of GRM8 in normal breast tissues (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean GRM8 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal breast tissues, a *p*-value, listed above.