The gastrin releasing peptide, GRP, is differentially expressed in brain 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 gastrin releasing peptide, encoded by GRP, was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. Molecular functions of gastrin releasing peptide may be relevant to the processes by which tumor cells of the breast metastasize to the brain. Down-regulation of GRP may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, GRP, gastrin releasing peptide, 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 brain metastatic breast cancer^{4,5}. We discovered significant differential and decreased expression of GRP in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE52604⁵ 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. 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 GRP 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 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.

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

We identified the gastrin releasing peptide, encoded by GRP, 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, GRP ranked 40 out of 22297 total transcripts (Table 1), equating to 99.82% differential expression. Differential expression of GRP in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=3.64E-05$).

Cross-validation of differentially expressed genes across datasets in cancer can be difficult and more challenging than when cross-validating across organs and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked whether we could validate differential expression of GRP when comparing brain metastases from patients with breast cancer to normal breast tissue as opposed to primary tumors of the breast.

Comparison of global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁵ revealed that GRP was among the genes whose expression was most significantly different transcriptome-wide between the breast and brain metastases in patients with breast cancer (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, GRP ranked 1185 out of 41093 total transcripts (Table 2), equating to 97.1% differential expression. Differential expression of GRP in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=3.73E-07$).

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

We obtained exact mRNA expression levels for GRP, 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 GRP expression in brain metastatic tissues. GRP 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 ± 0.23 in GRP expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified GRP as among the genes whose expression was most different in the brain metastases of patients with breast cancer both when compared to primary tumors of the breast and to the normal breast; GRP was expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast.

Discussion

We provided evidence here that gastrin releasing peptide is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer. Gastrin releasing peptide may be of relevance to any number of steps required for metastasis to the brain.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
40	206326_at	3.64E-05	-4.7369339	2.184	0.62 ± 0.23	GRP	gastrin releasing peptide

Table 1: GRP 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 GRP 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
1185	A_23_P101131	3.73E-07	-5.9074073	6.348189	GRP	gastrin releasing peptide

Table 2: GRP 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, fold change (FC) of GRP expression between the brain metastases of patients with breast cancer and normal breast tissue, gene and gene name are listed in this chart.

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

The mRNA expression level of GRP 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 GRP 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.