GRB7 is differentially expressed in the lymph node and 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 growth factor receptor-bound protein 7 encoded by GRB7 was among the genes whose expression was most different in the brain and lymph node metastases of patients with metastatic breast cancer as compared to transformed and benign breast tissues, respectively. GRB7 messenger RNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Up-regulation of GRB7 may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer. These data combined allude to some level of common origin for metastases that reside in the lymph nodes and colonize the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, lymph node metastasis, GRB7, growth factor receptor-bound protein 7, 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 brain metastatic breast cancer^{4,5}. We discovered significant differential and increased expression of the growth factor receptor-bound protein 7, encoded by GRB7, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE124648⁵ and 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 $n=4$ brain metastases from patients with breast cancer; analysis performed using platform GPL885. 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 GRB7 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 and lymph node metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

GRB7 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through analysis of published microarray data⁴, we identified the growth factor receptor-bound protein 7, encoded by GRB7, 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, GRB7 ranked 75 out of 17418 total transcripts (Table 1), equating to 99.6% differential expression. Differential expression of GRB7 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=1.5E-04$).

GRB7 is differentially expressed in the lymph node metastases of patients with metastatic breast cancer.

Next, we queried a second microarray dataset⁵ generated using lymph node metastases and normal breast tissues from patients with breast cancer to determine whether we could validate differential expression of GRB7 in metastatic tissues of humans with breast cancer, and whether differential expression of GRB7 was specific to brain metastases or could be observed in metastases to other sites in patients with metastatic breast cancer. We observed significant differential expression of GRB7 in lymph node metastases of patients with metastatic breast cancer (Table 2). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues in patients with breast cancer, GRB7 ranked 6264 out of 22283 total transcripts (Table 2), equating to 71.9% differential expression. Differential expression of GRB7 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Table 2; $p=1.69E-02$). Thus, differential expression of GRB7 was conserved in the brain and lymph node metastases of patients with metastatic breast cancer in two independent microarray datasets^{4,5}.

GRB7 is expressed at higher levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for GRB7, 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 GRB7 expression in brain metastatic tissues. GRB7 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 trended towards statistical significance (Figure 1; $p=0.0690$). We calculated a mean fold change in GRB7 expression of 4.14 ± 2.27 when comparing brain metastases to primary tumors of the breast.

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified GRB7 as among the genes whose expression was most different, transcriptome-wide, in the brain and lymph node metastases of patients with breast cancer when compared to the transformed and benign breast, respectively; GRB7 expression, as compared to primary tumors of the breast, was higher in metastasis to the brain.

Discussion

We provided evidence here that growth factor receptor bound protein 7⁷, encoded by GRB7, is among the genes whose expression is most different in the brain and lymph node metastases of patients with brain metastatic brain cancer. Inhibition of GRB7 in triple negative breast cancer cell lines results in induction of cell death and impaired *in vitro* invasion⁷. Evaluation of the effects of genetic depletion of GRB7 in mouse models of metastatic breast cancer on metastasis to the central nervous system are merited. Differential expression of growth factor receptor bound protein 7 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, penetrate the central nervous system and subsequently colonize the brain.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
75	10859	1.5E-04	-4.95	1.156977	4.14 ± 2.27	GRB7	growth factor receptor-bound protein 7

Table 1: GRB7 is differentially expressed in brain metastases in 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
6264	210761_s_at	1.69E-02	2.462639	-4.065857	GRB7	growth factor receptor bound protein 7

Table 2: GRB7 is differentially expressed in lymph node metastases of patients with 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: GRB7 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 GRB7 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 GRB7 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.