Differential expression of the eukaryotic translation initiation factor EIF1B in the brain metastases of patients with breast cancer.

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Metastasis to the brain is a complication of breast cancer^{1,2} with limited treatment options³. We mined published microarray data^{4,5} to discover genes associated with brain metastases in patients with metastatic breast cancer. We identified significant differential expression of the eukaryotic translation initiation factor EIF1B in the brain metastases of patients with breast cancer as compared to primary tumors of the breast. EIF1B may be relevant to the underlying biology by which tumor cells of the breast spread to the brain.

Keywords: EIF1B, breast cancer, brain metastases, central nervous system metastases, systems biology of breast cancer, targeted therapeutics in breast cancer.

One study documented a 34% incidence of metastases to the central nervous system in breast cancer patients treated with trastuzumab⁶. An unbiased and systems-level understanding of the transcriptional makeup of brain metastatic tissues can support identification of therapeutic targets. We performed global transcriptional profiling of brain metastases in patients with brain metastatic breast cancer⁶⁻⁸. We discovered significant differential expression of the eukaryotic translation initiation factor EIF1B 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 EIF1B 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 sought to discover genes associated with metastasis to the brain in patients with breast cancer by mining published microarray datasets^{4,5}, comparing global gene expression profiles of primary tumors of the breast to brain metastatic tissues from patients with breast cancer.

EIF1B is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the gene encoding the eukaryotic translation initiation factor 1B, EIF1B, as among the genes whose expression was most quantitatively different in brain metastases when compared to primary tumors of the breast in patients with breast cancer⁴. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, EIF1B ranked 313 out of 22277 total transcripts (Table 1), equating to 98.6% differential expression. Differential expression of EIF1B in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=3.62E-03$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of EIF1B in brain metastatic breast cancer. We again found that EIF1B was among the genes whose expression was most significantly different when comparing primary tumors of the breast to brain metastases from patients with metastatic breast cancer. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, EIF1B ranked 7220 out of 61359 total transcripts (Table 2), equating to 88.2% differential expression. Differential expression of EIF1B in brain metastases in patients with breast cancer approached the level of statistical significance (Table 2; $p=0.05654429$).

EIF1B is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

We obtained exact mRNA expression levels for EIF1B in primary tumors of the breast and in brain metastases to evaluate magnitude and direction of change in EIF1B gene expression in brain metastatic tissues. In both datasets queried, EIF1B was expressed at higher levels in brain metastases from patients with breast cancer as compared to primary tumors of the breast (Figure 1 and Figure 2). Increased expression of EIF1B in brain metastases as compared to primary tumors of the breast was statistically significant (Figure 1: $p=0.0016$). We calculated a mean fold change of 1.19 ± 0.11 in EIF1B expression in brain metastases when compared to primary tumors of the breast (Table 1).

Thus, through comparative transcriptome analysis of tumor and metastatic tumor tissues of patients with breast cancer using published microarray data^{4,5}, we found that EIF1B was among the genes most differentially expressed in brain metastases, with significantly increased expression in brain metastatic tissues as compared to primary tumors of the breast.

Discussion

To facilitate discovery of novel therapeutic targets in brain metastatic breast cancer, and to more thoroughly understand the biology of metastasis to the brain in breast cancer, we mined published microarray data^{4,5} to identify genes most differentially expressed in the brain metastases of patients with metastatic breast cancer.

Translation takes place in three broad steps: initiation, elongation and termination⁷. Eukaryotic initiation factors, EIFs, function in translational initiation. EIF1B is a paralog of EIF1 with which it shares 92% homology⁸. The function of EIF1B in translation initiation is not yet understood^{8,9}. EIF1 and EIF1B appear to exist in an antagonistic relationship in some contexts as depletion of EIF1 in the HAP1 chronic myelogenous leukemia (CML) cell line results in transcriptional induction of EIF1B⁹. However, EIF1B was dispensable for proliferation, both in HAP1 cells and in the HCT116 colorectal cancer cell line⁹.

Future studies can assess whether translation initiation is perturbed in brain metastases and whether brain metastatic tissues display any vulnerabilities to inhibition of translation machinery including EIF1B. We found here using published microarray data^{4,5} that EIF1B is among the genes whose expression changes most significantly in the brain metastases of patients with metastatic breast cancer and present at significantly higher quantities at the mRNA level in brain metastatic tissue.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
313	201738_at	3.62E-03	3.1226903	-1.8566	1.19 ± 0.11	EIF1B	eukaryotic translation initiation factor 1B

Table 1: Differential expression of EIF1B in brain metastases in patients with 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 EIF1B 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
7220	Hs. 315230.0.A1_3p_at	0.05654429	-1.9597964	-3.97958	EIF1B	eukaryotic translation initiation factor 1B

Table 2: Differential expression of EIF1B in brain metastases in patients with 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: EIF1B is expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast in patients with brain metastatic breast cancer.

The mRNA expression level of EIF1B 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 EIF1B 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.