Hexokinase 2 (HK2) is over-expressed 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 hexokinase 2, HK2, a metabolic enzyme converting glucose to glucose-6-phosphate, among the genes most differentially expressed in the brain metastases of patients with brain metastatic breast cancer. We recently reported that aldolase B is differentially expressed in metastatic brain tissues in human breast cancer⁶. Thus, genes involved in both fructose and glucose metabolism are among those most significantly perturbed at the transcriptional level in the brain metastases of humans with metastatic breast cancer.

Keywords: breast cancer, brain metastases, central nervous system metastases, HK2, hexokinase 2, 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. 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 over-expression of hexokinase 2 in brain metastases from patients with brain 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 HK2 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.

HK2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the gene encoding hexokinase 2, HK2, 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, HK2 ranked 308 out of 22297 total transcripts (Table 1), equating to 98.6% differential expression. Differential expression of HK2 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=3.51E-03$).

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 HK2 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 HK2 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, HK2 ranked 3707 out of 41093 total transcripts (Table 2), equating to 91.0% differential expression. Differential expression of HK2 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=5.63E-05$).

HK2 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for HK2, 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 HK2 expression in brain metastatic tissues. HK2 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; $p=0.0023$). We calculated a mean fold change of 1.19 ± 0.09 in HK2 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 hexokinase 2 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; HK2 was expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast.

Discussion

Hexokinase 2, like other hexokinases, functions in the conversion of glucose to glucose-6-phosphate by phosphorylation using ATP⁶. HK2 is over-expressed in a cancer of the brain, glioblastoma multiforme, and ectopic expression of HK2 results in enhancement of both GBM cell proliferation and intracranial growth of U87 GBM cells in NODSCID mice⁸. Importantly, in an ERBB2-driven mouse model of breast cancer, HK2 is required for both initiation and maintenance of tumors⁹. One previous study has documented increased expression of HK2 in laser-captured epithelial cells from brain metastases in patients with breast cancer; high HK2 expression was associated with inferior patient survival following craniotomy¹⁰. In pancreatic ductal adenocarcinoma (PDAC), HK2 is expressed at higher levels in primary tumors when compared to normal pancreas, and even higher levels in metastases when compared to primary PDAC tumor tissue¹¹. In CFPAC-1 and PANC-1 PDAC cell lines, HK2 was important for both anchorage independent growth and for *in vitro* migration¹². Depletion of HK2 significantly reduced the production of lactate concomitant with impairment of *in vitro* transwell migration¹². HK2 is important in multiple cancer types at differing stages of tumorigenesis, from tumor initiation to metastasis.

We report here that hexokinase 2 is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. We recently described the differential expression and up-regulation of aldolase B, an enzyme functioning in fructose metabolism, in the brain metastases of patients with breast cancer⁶. Thus, enzymes involved in both glucose and fructose metabolism are among the most significant transcriptional features of brain metastatic breast cancer.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
308	202934_at	3.51E-03	3.1339285	-1.8309	1.19 ± 0.09	HK2	hexokinase 2

Table 1: HK2 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 HK2 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
3707	A_32_P175739	5.63E-05	4.4293394	1.504152	HK2	hexokinase 2

Table 2: HK2 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: Hexokinase 2 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of hexokinase 2 (HK2) 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 HK2 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.