Fam174B is a differentially expressed gene in brain metastatic human 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 for the discovery of genes associated with brain metastasis in humans with metastatic breast cancer. We found that family with sequence similarity 174 member B, encoded by Fam174B, also known as LOC400451, 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 breast⁴. Fam174B mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of Fam174B in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of Fam174B expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, family with sequence similarity 174 member B, Fam174B, LOC400451, 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 trastuzumab⁶. 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 differential and increased expression of the gene encoding family with sequence similarity 174 member B, Fam174B, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE100534⁵ 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 metastatic breast cancer; analysis was performed using platform GPL885. GSE100534 was generated using Affymetrix Human Gene 1.0 ST Array with $n=16$ primary breast tumors and $n=3$ brain metastases from patients metastatic breast cancer; analysis was performed using platform GPL6244. 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 Fam174B gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of Fam174B mRNA expression levels with recurrence-free survival in $n=3951$ breast cancer patients, and with distant metastasis-free survival in $n=1803$ breast cancer patients.

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.

Fam174B is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified

family with sequence similarity 174 member B, encoded by Fam174B, also known as LOC400451, 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, Fam174B ranked 141 out of 17418 total transcripts (Chart 1), equating to 99.2% differential expression. Differential expression of Fam174B in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=3.15E-04$).

To attempt to validate transcriptome-wide differential expression of Fam174B in brain metastatic tissues of patients with metastatic breast cancer, we queried a second microarray dataset⁵, here comparing primary tumors from patients with breast cancer to brain metastases. Again, we identified Fam174B as a differentially expressed gene in human brain metastatic breast cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast, Fam174B ranked 6660 out of 33297 total transcripts (Chart 2), equating to 80.0% differential expression. Differential expression of Fam174B in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=3.27E-02$). Thus, differential expression of Fam174B, transcriptome-wide, in brain metastatic tissues of patients with metastatic breast cancer could be observed across two independent microarray datasets.

Fam174B is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for Fam174B, 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 Fam174B expression in brain metastatic tissues. Fam174B 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; $p=0.000909$).

Fam174B expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in 3951 breast cancer patients in total, to evaluate whether Fam174B tumor expression was correlated with survival outcomes in human breast cancer. We observed a statistically significant correlation between primary tumor expression of Fam174B and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of Fam174B possessed median RFS of 37 months, while patients whose tumors expressed high levels of Fam174B possessed median RFS of 68.48 months. This difference in RFS based on Fam174B tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: $9e-11$; hazard ratio: 0.7 (0.62-0.78)).

1 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
2 identified family with sequence similarity 174 member B, encoded by Fam174B, as among the
3 genes whose expression was most different, transcriptome-wide, in the brain metastases of
4 patients with breast cancer, when compared to primary tumors of the breasts; we observed
5 significantly increased expression of Fam174B in brain metastases as compared to primary
6 tumors of the breast. Further, we found a significant correlation between Fam174B expression
and patient survival outcomes, as recurrence-free survival was significantly greater in patients
whose primary tumors expressed higher levels of Fam174B as compared to patients whose
primary tumors expressed lower levels of Fam174B.

7 **Discussion**

8 We provided evidence here that the family with sequence similarity 174 member B,
9 encoded by Fam174B, is among the genes whose expression is most different in the brain
10 metastases of patients with brain metastatic breast cancer, that Fam174B mRNA is present at
11 significantly increased quantities in brain metastatic tissues as compared to primary tumors of the
12 breast, and that primary tumor Fam174B expression is significantly correlated with patient
13 survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of
14 Fam174B in mouse models of metastatic breast cancer on metastasis to the central nervous
15 system is merited. Modulation of Fam174B expression may be relevant to the processes by
16 which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the
17 lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.
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Rank: 141
Probe ID: 14282
P-value: 3.15E-04
t: -4.59
B: 0.47321
Gene: LOC400451
Gene name: hypothetical gene supported by AK075564; BC060873

Chart 1: Fam174B is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

The rank of global differential expression, the probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 6660
probe ID: 7991453
p-value: 3.27E-02
t: -2.2934459
B: -4.086377
Gene: Fam174B
Gene name: family with sequence similarity 174 member B

Chart 2: Fam174B is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: Fam174B is expressed at significantly higher levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of Fam174B in primary tumors of the breast (left) and in brain metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in Fam174B expression between primary tumors and brain metastasis, a p -value, is $p=0.000909$.

Figure 2: Significant correlation between Fam174B primary tumor expression and recurrence-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=3951$ total patients stratified into two groups, based on low or high expression of Fam174B in patient primary tumors. The log rank p -value denoting statistical significance of difference in recurrence-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on Fam174B expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low Fam174B expression: 37 months
High Fam174B expression: 68.48 months

Chart 3: Median recurrence-free survival is inferior in patients with high primary tumor expression of Fam174B.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of Fam174B and high primary tumor expression of Fam174B is listed in this chart, for $n=3951$ breast cancer patients.