FICD 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 the protein adenylyltransferase FICD, encoded by FICD, 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, as well as in primary tumors of the breast when compared to normal breast tissue⁵. FICD mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of FICD in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of FICD 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, protein adenylyltransferase FICD, FICD, 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 protein adenylyltransferase FICD, FICD, in brain metastatic tissues of patients with metastatic breast cancer, and in the primary tumors of patients with breast cancer.

Methods

We used datasets GSE10893⁴ and GSE109169⁵ 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. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array with $n=25$ normal breast tissues and $n=25$ primary breast tumors; analysis was performed using platform GPL5175. 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 FICD 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 FICD mRNA expression levels with recurrence-free survival in $n=3951$ 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.

FICD 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 the

protein adenylyltransferase FICD, encoded by FICD, 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, FICD ranked 95 out of 17418 total transcripts (Chart 1), equating to 99.5% differential expression. Differential expression of FICD in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.64\text{e-}04$).

To attempt to validate transcriptome-wide differential expression of FICD in humans with breast cancer, we queried a second microarray dataset⁵, here comparing primary tumors from patients with breast cancer to normal breast tissue. Again, we identified FICD as a differentially expressed gene in human breast cancer; here, in primary tumors of the breast (Chart 2). When sorting each of the genes expressed in primary tumors of the breast based on significance of difference as compared to normal breast tissue, FICD ranked 4583 out of 19076 total transcripts (Chart 2), equating to 76.0% differential expression. Differential expression of FICD in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=2.75\text{e-}04$). Thus, differential expression of FICD, transcriptome-wide, in brain metastatic tissues of patients with metastatic breast cancer could be observed across two independent microarray datasets.

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

We obtained exact mRNA expression levels for FICD, 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 FICD expression in brain metastatic tissues. We observed significantly lower expression of FICD in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: FICD was expressed at -0.01 ± 0.25 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.95 ± 0.41 AU in brain metastatic tissues (Figure 1). The difference in FICD mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000209$).

FICD 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 FICD tumor expression was correlated with survival outcomes in human breast cancer. We observed a statistically significant correlation between primary tumor expression of FICD and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of FICD possessed median RFS of 39 months, while patients whose tumors expressed high levels of FICD possessed median RFS of 59 months. This difference in RFS based on FICD tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.00019; hazard ratio: 0.81 (0.73-0.91)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we

1 identified the protein adenylyltransferase FICD, encoded by FICD, as among the genes whose
2 expression was most different, transcriptome-wide, in the brain metastases of patients with breast
3 cancer, when compared to primary tumors of the breasts; we observed significantly decreased
4 expression of FICD in brain metastases as compared to primary tumors of the breast. Further, we
5 found a significant correlation between FICD expression and patient survival outcomes, as
6 recurrence-free survival was significantly greater in patients whose primary tumors expressed
7 higher levels of FICD as compared to patients whose primary tumors expressed lower levels of
8 FICD.

9 **Discussion**

10 We provided evidence here that the protein adenylyltransferase FICD, encoded by FICD, is
11 among the genes whose expression is most different in the brain metastases of patients with brain
12 metastatic breast cancer, that FICD mRNA is present at significantly decreased quantities in brain
13 metastatic tissues as compared to primary tumors of the breast, and that primary tumor FICD
14 expression is significantly correlated with patient survival outcomes in human breast cancer.
15 FICD and the post-translational modification it catalyzes, AMPylation, has recently been
16 described to promote the differentiation of neural progenitor stem cells⁸. Evaluation of the effects
17 of genetic depletion of FICD in mouse models of metastatic breast cancer on metastasis to the
18 central nervous system is merited. Modulation of FICD expression may be relevant to the
19 processes by which breast cancer cells exit the breast, enter the vasculature and/or lymphatics,
20 reside in the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize
21 the brain.
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Probe ID: 13736
P-value: 2.64e-04
t: -4.76
B: 0.657
Gene: FICD
Gene name: protein adenylyltransferase FICD

Chart 1: FICD 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.

probe ID: 3430716

P-value: 2.75e-04

t: -3.91

B: -0.243294

Gene: FICD

Gene name: protein adenylyltransferase FICD

Chart 2: FICD is differentially expressed in primary tumors of the breast when compared to normal breast tissue.

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: FICD is expressed at significantly lower levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of FICD 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 FICD expression between primary tumors and brain metastasis, a *p*-value, is *p*=0.000209.

Figure 2: Significant correlation between FICD 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 FICD 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 FICD 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.

High FICD expression: 59 months

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

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