The interferon-related developmental regulator 1 is a differentially expressed gene in both lymph node and brain metastases in 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 interferon-related developmental regulator 1, encoded by IFRD1, was among the genes whose expression was most quantitatively different in the brain metastases of patients with metastatic breast cancer. IFRD1 mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of IFRD1 in primary tumors was significantly correlated with patient overall survival in patients with breast cancer. Modulation of IFRD1 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, interferon-related developmental regulator 1, IFRD1, 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 decreased expression of the gene encoding interferon-related developmental regulator 1, IFRD1, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE124648⁵ 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 was performed using platform GPL885. GSE124648 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; analysis was performed using platform GPL96. 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 IFRD1 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 IFRD1 mRNA expression levels in primary tumors of the breast with overall survival in $n=1402$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic and primary tumor tissues of patients with metastatic breast, as well as normal tissues of the breast 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.

IFRD1 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 interferon-related developmental regulator 1, encoded by IFRD1, as a differentially expressed gene in the brain 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, IFRD1 ranked 98 out of 17418 total transcripts (Chart 1), equating to 99.4% differential expression. Differential expression of IFRD1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.93e-04$).

IFRD1 is differentially expressed in the lymph node metastases of patients with breast cancer.

To determine whether differential expression of IFRD1 in human breast cancer could be validated in a second microarray dataset, and whether this was specific to brain metastases or could be observed in other anatomical locations, we queried a second microarray dataset⁵, here comparing normal breast tissues to lymph node metastases. Again, we identified IFRD1 as a differentially expressed gene in the lymph node metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues, IFRD1 ranked 5334 out of 22283 total transcripts (Chart 2), equating to 76.1% differential expression. Differential expression of IFRD1 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=8.63e-03$). Thus, differential expression of IFRD1, transcriptome-wide, in metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets, in the brain and in the lymph nodes.

IFRD1 is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for IFRD1, 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 IFRD1 expression in brain metastatic tissues. We observed significantly decreased expression of IFRD1 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: IFRD1 was expressed at -0.85 ± 0.46 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -2.04 ± 0.27 AU in brain metastatic tissues (Figure 1). The difference in IFRD1 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000339$).

IFRD1 expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in 1402 breast cancer patients in total, to evaluate whether IFRD1 tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of IFRD1 and overall survival (OS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of IFRD1 possessed median OS of 120 months, while patients whose tumors expressed high levels of IFRD1 possessed median OS of 84 months. This difference in OS based on IFRD1 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value 0.029; hazard ratio: 1.27(1.02-1.57)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified interferon-related developmental regulator 1, encoded by IFRD1, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer, when compared to primary tumors of the breast and to normal breast tissues, respectively; we observed significantly decreased expression of IFRD1 in brain metastases as compared to primary tumors of the breast. Further, we found a correlation between IFRD1 expression and patient survival outcomes, as overall survival was greater in patients whose primary tumors expressed higher levels of IFRD1 as compared to patients whose primary tumors expressed lower levels of IFRD1.

Discussion

We provided evidence here that IFRD1 is among the genes whose expression is most different in the brain metastases of patients with metastatic breast cancer, that IFRD1 mRNA is present at significantly decreased quantity in brain metastatic tissues as compared to primary tumors of the breast, that IFRD1 was also differentially expressed in the lymph nodes, and that primary tumor IFRD1 expression is correlated with patient survival outcomes in human breast cancer. It appears that IFRD1 is a regulator of NF- κ B pathway but whether it activates or represses signal transduction is not clear^{8,9}. Evaluation of the effects of genetic depletion of IFRD1 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of IFRD1 expression may be relevant to the processes by which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.

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Rank: 98
Probe ID: 5825
p-value: 1.93e-04
t: 4.83
B: 0.922193
Gene: IFRD1
Gene name: interferon-related developmental regulator 1

Chart 1: IFRD1 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: 5334
probe ID: 202147_s_at
p-value: 8.63e-03
t: 2.723161
B: -3.458594
Gene: IFRD1
Gene name: interferon-related developmental regulator 1

Chart 2: IFRD1 is differentially expressed in the lymph node metastases of patients with breast cancer when comparing lymph node metastases to normal breast tissues.

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: IFRD1 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 IFRD1 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 IFRD1 expression between primary tumors and brain metastases is $p=0.000339$.

Figure 2: IFRD1 primary tumor expression is correlated with overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total breast cancer patients stratified into two groups, based on low or high expression of IFRD1 in patient primary tumor. The log rank p -value denoting statistical significance of difference in overall 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 IFRD1 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 IFRD1 expression: 120 months
High IFRD1 expression: 84 months

Chart 3: Median overall survival is inferior in patients with high primary tumor expression of IFRD1.

The median OS (overall survival) of patients with low primary tumor expression of IFRD1 and high primary tumor expression of IFRD1 is listed in this chart, for $n=1402$ breast cancer patients.