Fam158a (EMC9) 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 158, member A, also known as EMC9 and C14orf122, encoded by Fam158a, 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 breast. Fam158a mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of Fam158a in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of Fam158a 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, metastatic breast cancer, metastasis, brain metastasis, central nervous system metastasis, family with sequence similarity 158, member A, Fam158a, EMC9, C14orf122, 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 158, member A, Fam158a, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE43837⁵ 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 breast cancer; analysis was performed using platform GPL885. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary tumors of the breast and $n=19$ brain metastases from patients with breast cancer; analysis was 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 Fam158a 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 Fam158a 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.

Fam158a 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 158, member A, also known as EMC9 and C14orf122, encoded by

Fam158a, 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, Fam158a ranked 64 out of 17418 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of Fam158a in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.21\text{E-}04$).

To attempt to validate differential transcriptome-wide differential expression of Fam158a in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵; again, we identified Fam158a as a differentially expressed gene in the brain metastatic tissues of patients with 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, Fam158a ranked 6637 out of 61359 total transcripts (Chart 2), equating to 89.2% differential expression. Differential expression of Fam158a in the brain metastases of patients with metastatic breast cancer approached the level of statistical significance (Chart 2; $p=0.05107315$). Thus, transcriptome-wide differential expression of Fam158a in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, indicating that this may likely be a *bona fide* biological phenomena associated with metastatic breast cancer in humans.

Fam158a is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for Fam158a, 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 Fam158a expression in brain metastatic tissues. Fam158a 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; $p=0.000314$).

Fam158a expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in 3951 breast cancer patients in total, to evaluate whether Fam158a tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of Fam158a and recurrence-free survival (RFS) for patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of Fam158a possessed median RFS of 216.66 months, while patients whose tumors expressed high levels of Fam158a possessed median RFS of 171.43 months. This difference in RFS based on Fam158a tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: $9.2\text{e-}07$; hazard ratio: 1.31 (1.18-1.47)).

1 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
2 identified family with sequence similarity 158, member A, encoded by Fam158a, 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 breast; we observed
5 significantly increased expression of Fam158a in brain metastases as compared to primary tumors
6 of the breast. Further, we found a significant correlation between Fam158a expression and
patient survival outcomes, as recurrence-free survival was significantly higher in patients whose
primary tumors expressed lower levels of Fam158a as compared to patients whose primary
tumors expressed higher levels of Fam158a.

7 **Discussion**

8 We provided evidence here that family with sequence similarity 158, member A, encoded
9 by Fam158a, is among the genes whose expression is most different in the brain metastases of
10 patients with brain metastatic breast cancer, that Fam158a mRNA is present at significantly
11 increased quantities in brain metastatic tissues as compared to primary tumors of the breast, and
12 that primary tumor Fam158a expression is significantly correlated with patient survival outcomes
in human breast cancer. Evaluation of the effects of genetic depletion of Fam158a in mouse
models of metastatic breast cancer on metastasis to the central nervous system is merited.
Modulation of Fam158a expression may be relevant to the processes by which breast cancer cells
13 exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune
14 clearance, breach the blood-brain barrier and colonize the brain.
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Rank: 64
Probe ID: 12485
p-value: 1.21E-04
t: -5.06
B: 1.353675
Gene: Fam158a
Gene name: family with sequence similarity 158, member A

Chart 1: Fam158a 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: 6637
probe ID: g7705615_3p_at
p-value: 0.05107315
t: -2.0071051
B: -3.90435
Gene: EMC9
Gene name: ER membrane protein complex subunit 9

Chart 2: Fam158a, also known as EMC9, 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 2: Significant correlation between Fam158a 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 Fam158a 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 Fam158a 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 Fam158a expression: 216.66 months
High Fam158a expression: 171.43 months

Chart 3: Median recurrence-free survival is inferior in patients with high primary tumor expression of Fam158a.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of Fam158a and high primary tumor expression of Fam158a is listed in this chart, for $n=3951$ breast cancer patients.