FAM3B is a differentially expressed gene in brain metastatic human breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 3, member B, encoded by FAM3B, 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. FAM3B mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of FAM3B in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of FAM3B 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 3, member B, FAM3B, 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 3, member B, FAM3B, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE52604⁵ 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. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ 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 FAM3B 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 FAM3B 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.

FAM3B 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

family with sequence similarity 3, member B, encoded by FAM3B, 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, FAM3B ranked 42 out of 17418 total transcripts (Chart 1), equating to 99.8% differential expression. Differential expression of FAM3B in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=8.30E-05$).

Differential gene expression analyses that utilize primary and metastatic tumor tissues to discover genes associated with metastasis can be challenging due to the relatively heterogeneous cellular composition and constitution of the tumor as compared to normal, untransformed (benign) tissues. Thus, to attempt to validate differential transcriptome-wide differential expression of FAM3B in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues rather than primary tumors of the breast to brain metastases. Again, we identified FAM3B 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 normal breast tissues, FAM3B ranked 5306 out of 41093 total transcripts (Chart 2), equating to 87.1% differential expression. Differential expression of FAM3B in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=2.97E-04$). Thus, differential expression of FAM3B, transcriptome-wide, in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, both when compared to benign and transformed breast tissues. This suggests that FAM3B differential expression in human brain metastatic breast cancer is a *bona fide* biological process.

FAM3B is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for FAM3B, 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 FAM3B expression in brain metastatic tissues. We observed significantly higher expression of FAM3B in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: FAM3B was expressed at -0.02 ± 0.73 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 2.94 ± 1.56 AU in brain metastatic tissues. The difference in FAM3B mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000337$).

FAM3B expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=3951$ breast cancer patients in total, to evaluate whether FAM3B tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of FAM3B and recurrence-free survival (RFS) in patients with breast cancer, in the lower survival quartile (Figure 2). Patients whose primary tumors expressed low levels of FAM3B possessed median RFS of 28.62 months, while patients whose tumors expressed high levels of FAM3B

possessed median RFS of 37.2 months. This difference in RFS based on FAM3B tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.04; hazard ratio: 0.83 (0.7-0.99)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified family with sequence similarity 3, member B, encoded by FAM3B, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer, both when compared to primary tumors of the breast and to normal breast tissues; we observed significantly increased expression of FAM3B in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between FAM3B expression and patient survival outcomes, as recurrence-free survival was significantly greater in patients whose primary tumors expressed higher levels of FAM3B as compared to patients whose primary tumors expressed lower levels of FAM3B.

Discussion

We provided evidence here that family with sequence similarity 3, member B, encoded by FAM3B, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that FAM3B mRNA is present at significantly increased quantities in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor FAM3B expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of FAM3B in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of FAM3B 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.

References

1. Lin, N.U., Amiri-Kordestani, L., Palmieri, D., Liewehr, D.J. and Steeg, P.S., 2013. CNS metastases in breast cancer: old challenge, new frontiers.
2. Bendell, J.C., Domchek, S.M., Burstein, H.J., Harris, L., Younger, J., Kuter, I., Bunnell, C., Rue, M., Gelman, R. and Winer, E., 2003. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer*, 97(12), pp.2972-2977.
3. Tsukada, Y., Fouad, A., Pickren, J.W. and Lane, W.W., 1983. Central nervous system metastasis from breast carcinoma autopsy study. *Cancer*, 52(12), pp.2349-2354.
4. Weigman, V.J., Chao, H.H., Shabalin, A.A., He, X., Parker, J.S., Nordgard, S.H., Grushko, T., Huo, D., Nwachukwu, C., Nobel, A. and Kristensen, V.N., 2012. Basal-like Breast cancer DNA copy number losses identify genes involved in genomic instability, response to therapy, and patient survival. *Breast cancer research and treatment*, 133(3), pp.865-880.
5. Salhia, B., Kiefer, J., Ross, J.T., Metapally, R., Martinez, R.A., Johnson, K.N., DiPerna, D.M., Paquette, K.M., Jung, S., Nasser, S. and Wallstrom, G., 2014. Integrated genomic and epigenomic analysis of breast cancer brain metastasis. *Plos one*, 9(1), p.e85448.
6. Awada, A., Colomer, R., Inoue, K., Bondarenko, I., Badwe, R.A., Demetriou, G., Lee, S.C., Mehta, A.O., Kim, S.B., Bachelot, T. and Goswami, C., 2016. Neratinib plus paclitaxel vs trastuzumab plus paclitaxel in previously untreated metastatic ERBB2-positive breast cancer: the NEfERT-T randomized clinical trial. *JAMA oncology*, 2(12), pp.1557-1564.
7. Györfy, B., Lanczky, A., Eklund, A.C., Denkert, C., Budczies, J., Li, Q. and Szallasi, Z., 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment*, 123(3), pp.725-731.

Rank: 42
Probe ID: 6567
p-value: 8.30E-05
t: -5.37
B: 1.7116
Gene: FAM3B
Gene name: family with sequence similarity 3, member B

Chart 1: FAM3B 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: 5306
probe ID: A_23_P166269
p-value: 2.97E-04
t: 3.908674
B: -0.081478
Gene: FAM3B
Gene name: family with sequence similarity 3, member B

Chart 2: FAM3B is differentially expressed in brain metastatic breast cancer when comparing brain 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.

FAM3B

Figure 1: FAM3B 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 FAM3B 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 FAM3B expression between primary tumors of the breast and brain metastases, a *p*-value, is $p=0.000337$.

Figure 2: Significant correlation between FAM3B 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 FAM3B in patient primary tumors, in the lower survival quartile. 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 FAM3B 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 FAM3B expression: 28.62 months

High FAM3B expression: 37.2 months

Chart 3: Median recurrence-free survival is inferior in patients with low primary tumor expression of FAM3B.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of FAM3B and high primary tumor expression of FAM3B is listed in this chart, for $n=3951$ breast cancer patients, in the lower survival quartile.