GRINL1A 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 the glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A, encoded by GRINL1A, 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. GRINL1A mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of GRINL1A in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of GRINL1A 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, glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A, GRINL1A, 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 glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A, GRINL1A, 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 $n=4$ 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 GRINL1A 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 GRINL1A 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.

GRINL1A 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

glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A, encoded by GRINL1A, 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, GRINL1A ranked 248 out of 17418 total transcripts (Chart 1), equating to 98.6% differential expression. Differential expression of GRINL1A in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=7.34e-04$).

To attempt to validate transcriptome-wide differential expression of GRINL1A 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 GRINL1A 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, GRINL1A ranked 1191 out of 19076 total transcripts (Chart 2), equating to 93.8% differential expression. Differential expression of GRINL1A in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=1.83e-08$). Thus, differential expression of GRINL1A, transcriptome-wide, in brain metastatic tissues of patients with metastatic breast cancer could be observed across two independent microarray datasets.

GRINL1A is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for GRINL1A, 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 GRINL1A expression in brain metastatic tissues. We observed significantly lower expression of GRINL1A in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: GRINL1A was expressed at -0.61 ± 0.45 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -1.74 ± 0.53 AU in brain metastatic tissues (Figure 1). The difference in GRINL1A mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.001273$).

GRINL1A 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 GRINL1A tumor expression was correlated with survival outcomes in human breast cancer. We observed a statistically significant correlation between primary tumor expression of GRINL1A and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of GRINL1A possessed median RFS of 41.95 months, while patients whose tumors expressed high levels of GRINL1A possessed median RFS of 61 months. This difference in RFS based on GRINL1A tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: $6.5e-7$; hazard ratio: 0.76 (0.68-0.85)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A, encoded by GRINL1A, 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 breasts; we observed significantly decreased expression of GRINL1A in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between GRINL1A expression and patient survival outcomes, as recurrence-free survival was significantly greater in patients whose primary tumors expressed higher levels of GRINL1A as compared to patients whose primary tumors expressed lower levels of GRINL1A.

Discussion

We provided evidence here that the glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A, encoded by GRINL1A, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that GRINL1A mRNA is present at significantly decreased quantities in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor GRINL1A expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of GRINL1A in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of GRINL1A 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. Schulten, H.J., Bangash, M., Karim, S., Dallol, A., Hussein, D., Merdad, A., Al-Thoubaity, F.K., Al-Maghrabi, J., Jamal, A., Al-Ghamdi, F. and Choudhry, H., 2017. Comprehensive molecular biomarker identification in breast cancer brain metastases. *Journal of translational medicine*, 15(1), p.269.
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: 248

Probe ID: 2243

P-value: 7.34e-04

t: 4.18

B: -0.307888

Gene: GRINL1A

Gene name: glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A

Chart 1: GRINL1A 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: 1191

probe ID: 3595441

P-value: 1.83e-08

t: 6.67

B: 9.116693

Gene: GRINL1A

Gene name: glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A

Chart 2: GRINL1A 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: GRINL1A 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 GRINL1A 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 GRINL1A expression between primary tumors and brain metastasis, a p -value, is $p=0.001273$.

Figure 2: Significant correlation between GRINL1A 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 GRINL1A 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 GRINL1A 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 GRINL1A expression: 41.95 months

High GRINL1A expression: 61 months

Chart 3: Median recurrence-free survival is inferior in patients with low primary tumor expression of GRINL1A.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of GRINL1A and high primary tumor expression of GRINL1A is listed in this chart, for $n=3951$ breast cancer patients.