GRHL3 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 grainyhead-like transcription factor 3, encoded by GRHL3, 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. GRHL3 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of GRHL3 in primary tumors was significantly correlated with patient overall survival. Modulation of GRHL3 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, grainyhead-like transcription factor 3, GRHL3, 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 grainyhead-like transcription factor 3, GRHL3, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE100534⁵ 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. GSE100534 was generated using Affymetrix Human Gene 1.0 ST Array with $n=16$ primary breast tumors and $n=3$ brain metastases from patients metastatic breast cancer; analysis was performed using platform GPL6244. 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 GRHL3 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 GRHL3 mRNA expression levels with overall survival in $n=626$ 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.

GRHL3 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

grainyhead-like transcription factor 3, encoded by GRHL3, 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, GRHL3 ranked 112 out of 17418 total transcripts (Chart 1), equating to 99.4% differential expression. Differential expression of GRHL3 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=3.64\text{e-}04$).

To attempt to validate transcriptome-wide differential expression of GRHL3 in brain metastatic tissues of patients with metastatic breast cancer, we queried a second microarray dataset⁵, here comparing primary tumors from patients with breast cancer to brain metastases. Again, we identified GRHL3 as a differentially expressed gene in human brain metastatic 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, GRHL3 ranked 2343 out of 41093 total transcripts (Chart 2), equating to 94.3% differential expression. Differential expression of GRHL3 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=2.19\text{e-}03$). Thus, differential expression of GRHL3, transcriptome-wide, in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets. This suggests that GRHL3 differential expression in human brain metastatic breast cancer is a *bona fide* biological process.

GRHL3 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for GRHL3, 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 GRHL3 expression in brain metastatic tissues. We observed significantly higher expression of GRHL3 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: GRHL3 was expressed at 0.15 ± 0.25 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.98 ± 0.13 AU in brain metastatic tissues. The difference in GRHL3 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000148$).

GRHL3 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=626$ breast cancer patients in total, to evaluate whether GRHL3 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of GRHL3 and overall survival (OS) in patients with breast cancer, in the lower survival quartile (Figure 2). Patients whose primary tumors expressed low levels of GRHL3 possessed median OS of 108 months, while patients whose tumors expressed high levels of GRHL3 possessed median OS of 68.4 months. This difference in OS based on GRHL3 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.022; hazard ratio: 1.57 (1.06-2.33)).

1 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
2 identified grainyhead-like transcription factor 3, encoded by GRHL3, as among the genes whose
3 expression was most different, transcriptome-wide, in the brain metastases of patients with breast
4 cancer when compared to primary tumors of the breast; we observed significantly increased
5 expression of GRHL3 in brain metastases as compared to primary tumors of the breast. Further,
6 we found a significant correlation between GRHL3 expression and patient survival outcomes, as
overall survival was significantly greater in patients whose primary tumors expressed lower
levels of GRHL3 as compared to patients whose primary tumors expressed higher levels of
GRHL3.

7 **Discussion**

8 We provided evidence here that grainyhead-like transcription factor 3, encoded by
9 GRHL3, is among the genes whose expression is most different in the brain metastases of
10 patients with brain metastatic breast cancer, that GRHL3 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 GRHL3 expression is significantly correlated with patient survival outcomes
in human breast cancer. Evaluation of the effects of genetic depletion of GRHL3 in mouse
models of metastatic breast cancer on metastasis to the central nervous system is merited.
13 Modulation of GRHL3 expression may be relevant to the processes by which breast cancer cells
14 exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune
15 clearance, breach the blood-brain barrier and colonize the brain.
16
17
18
19
20
21
22
23
24
25
26
27
28

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: 112
Probe ID: 16885
p-value: 3.64e-04
t: -4.60
B: 0.3626
Gene: GRHL3
Gene name: grainyhead-like transcription factor 3

Chart 1: GRHL3 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: 2343
probe ID: 7898916
p-value: 2.19e-03
t: -3.5106047
B: -1.580231
Gene: GRHL3
Gene name: grainyhead-like transcription factor 3

Chart 2: GRHL3 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 1: GRHL3 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 GRHL3 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 GRHL3 expression between primary tumors of the breast and brain metastases, a p -value, is $p=0.000148$.

Figure 2: Significant correlation between GRHL3 primary tumor expression and overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=626$ total patients stratified into two groups, based on low or high expression of GRHL3 in patient primary tumors, in the lower survival quartile. 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 GRHL3 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 GRHL3 expression: 108 months
High GRHL3 expression: 68.4 months

Chart 3: Median overall survival is inferior in patients with high primary tumor expression of GRHL3.

The median OS (overall survival) of patients with low primary tumor expression of GRHL3 and high primary tumor expression of GRHL3 is listed in this chart, for $n=626$ breast cancer patients, in the lower survival quartile.