GNB4 is a differentially expressed gene in human brain metastatic 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 guanine nucleotide-binding protein subunit beta-4, encoded by GNB4, was among the genes whose expression was most quantitatively different in the brain metastases of patients with metastatic breast cancer. GNB4 mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of GNB4 in primary tumors was significantly correlated with patient distant metastasis-free survival in patients with breast cancer. Modulation of GNB4 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, guanine nucleotide-binding protein subunit beta-4, GNB4, 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 guanine nucleotide-binding protein subunit beta-4, GNB4, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GS52604⁵ 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. 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 GNB4 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 GNB4 mRNA expression levels in primary tumors of the breast with distant metastasis-free survival in $n=664$ 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.

GNB4 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 guanine nucleotide-binding protein subunit beta-4, encoded by GNB4, 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, GNB4 ranked 117 out of 17418 total transcripts (Chart 1), equating to 99.3% differential expression. Differential expression of GNB4 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.32e-04$).

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 GNB4 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 GNB4 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, GNB4 ranked 4273 out of 41093 total transcripts (Chart 2), equating to 89.6% differential expression. Differential expression of GNB4 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=1.07\text{e-}04$). Thus, differential expression of GNB4, 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 GNB4 differential expression in human brain metastatic breast cancer is a *bona fide* biological process.

GNB4 is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for GNB4, 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 GNB4 expression in brain metastatic tissues. We observed significantly decreased expression of GNB4 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: GNB4 was expressed at 0.05 ± 0.63 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -1.58 ± 0.59 AU in brain metastatic tissues (Figure 1). The difference in GNB4 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000597$).

GNB4 expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=664$ breast cancer patients in total, to evaluate whether GNB4 tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of GNB4 and distant metastasis-free survival (DMFS) in patients with breast cancer (Figure 2). DMFS was greater in patients with low primary tumor expression of GNB4 as compared to patients with high primary tumor expression of GNB4. This difference in DMFS based on GNB4 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value 0.025; hazard ratio: 1.45 (1.05-2.01)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified guanine nucleotide-binding protein subunit beta-4, encoded by GNB4, 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 GNB4 in brain metastases as compared to primary tumors of the breast. Further, we found a correlation between GNB4 expression and patient survival outcomes, as distant metastasis-free survival was greater in patients whose primary tumors expressed lower levels of GNB4 as compared to patients whose primary tumors expressed higher levels of GNB4.

Discussion

We provided evidence here that GNB4 is among the genes whose expression is most different in the brain metastases of patients with metastatic breast cancer, that GNB4 mRNA is present at significantly decreased quantity in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor GNB4 expression is correlated with patient survival outcomes in human breast cancer. GNB4 is up-regulated during oligodendrocyte cell commitment and its ectopic expression can drive differentiation of oligodendrocyte progenitor cells to the oligodendrocyte fate⁸. Evaluation of the effects of genetic depletion of GNB4 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of GNB4 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.
8. Pol, S.U., Polanco, J.J., Seidman, R.A., O'Bara, M.A., Shayya, H.J., Dietz, K.C. and Sim, F.J., 2017. Network-based genomic analysis of human oligodendrocyte progenitor differentiation. *Stem cell reports*, 9(2), pp.710-723.

Rank: 117
Probe ID: 12531
p-value: 2.32e-04
t: 4.74
B: 0.752324
Gene: GNB4
Gene name: guanine nucleotide-binding protein subunit beta-4

Chart 1: GNB4 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: 4273
probe ID: A_23_P18186
p-value: 1.07e-04
t: -4.2318571
B: 0.89238
Gene: GNB4
Gene name: guanine nucleotide-binding protein subunit beta-4

Chart 2: GNB4 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.

Figure 1: GNB4 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 GNB4 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 GNB4 expression between primary tumors and brain metastases is $p=0.000597$.

Figure 2: GNB4 primary tumor expression is correlated with distant metastasis-free survival in patients with breast cancer, and distant metastasis-free survival is superior in patients with low primary tumor expression of GNB4.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival for $n=664$ total breast cancer patients stratified into two groups, based on low or high expression of GNB4 in patient primary tumor. The log rank p -value denoting statistical significance of difference in distant metastasis-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 GNB4 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.