Carbonic anhydrase VIII (CA8) 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 carbonic anhydrase VIII, encoded by CA8, 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. CA8 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of CA8 in primary tumors was significantly correlated with patient distant metastasis-free survival. Modulation of CA8 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, carbonic anhydrase 8, CA8, 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 carbonic anhydrase 8, CA8, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE72653⁵ 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. GSE72653 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=9$ normal epithelium and $n=9$ tumors of the breast 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 CA8 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 CA8 mRNA expression levels with distant metastasis-free survival in $n=1803$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of primary and metastatic tumor tissues of patients with breast cancer and 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.

CA8 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 carbonic anhydrase VIII, encoded by CA8, 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, CA8 ranked 11 out of 17418 total transcripts (Chart 1), equating to 99.9% differential expression. Differential expression of CA8 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=3.68E-06$).

To attempt to validate differential transcriptome-wide differential expression of CA8 in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues and primary tumors of the breast. Again, we identified CA8 as a differentially expressed gene in the tumor tissues of patients with breast cancer (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 tissues, CA8 ranked 9433 out of 41093 total transcripts (Chart 2), equating to 77.04% differential expression. Differential expression of CA8 in the primary tumors of patients with breast cancer was statistically significant (Chart 2; $p=4.13E-02$). Thus, differential expression of CA8, transcriptome-wide, in the primary tumors of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

CA8 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for CA8, 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 CA8 expression in brain metastatic tissues. CA8 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; $p<0.00001$).

CA8 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in $n=1803$ breast cancer patients in total, to evaluate whether CA8 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of CA8 and distant metastasis-free survival (DMFS) in patients with breast cancer in the lower survival quartile (Figure 2). Patients whose primary tumors expressed low levels of CA8 possessed median DMFS of 72 months, while patients whose tumors expressed high levels of CA8 possessed median RFS of 114.02 months. This difference in DMFS based on CA8 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.041; hazard ratio: 0.8 (0.65-0.99)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified carbonic anhydrase VIII, encoded by CA8, 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 CA8 in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between CA8 expression and patient

1 survival outcomes, as distant metastasis-free survival was significantly greater in patients whose
2 primary tumors expressed higher levels of CA8 as compared to patients whose primary tumors
3 expressed lower levels of CA8.

4 **Discussion**

5 We provided evidence here that carbonic anhydrase 8, encoded by CA8, is among the
6 genes whose expression is most different in the brain metastases and primary tumors of humans
7 with breast cancer, that CA8 mRNA is present at significantly increased quantities in brain
8 metastatic tissues as compared to primary tumors of the breast, and that primary tumor CA8
9 expression is significantly correlated with patient survival outcomes in human breast cancer.
10 Evaluation of the effects of genetic depletion of CA8 in mouse models of metastatic breast cancer
11 on metastasis to the central nervous system is merited. Modulation of CA8 expression may be
12 relevant to the processes by which breast cancer cells exit the breast, enter the vasculature and/or
13 lymphatics, reside in the lymph nodes, evade immune clearance, breach the blood-brain barrier
14 and colonize the brain.
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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: 11
Probe ID: 16234
p-value: 3.68E-06
t: -6.93
B: 4.488103
Gene: CA8
Gene name: carbonic anhydrase VIII

Chart 1: CA8 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: 9433
probe ID: A_23_P83838
p-value: 4.13E-02
t: -2.1888006
B: -3.974021
Gene: CA8
Gene name: carbonic anhydrase VIII

Chart 2: CA8 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: CA8 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 CA8 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 CA8 expression between primary tumors of the breast and brain metastases is listed above as a *p*-value.

Figure 2: Significant correlation between CA8 primary tumor expression and distant metastasis-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival for $n=1803$ total patients stratified into two groups, based on low or high expression of CA8 in patient primary tumors. 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 CA8 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 CA8 expression: 72 months
High CA8 expression: 114.02 months

Chart 3: Median distant metastasis-free survival is inferior in patients with low primary tumor expression of CA8.

The median DMFS (distant metastasis-free survival) of patients with low primary tumor expression of CA8 and high primary tumor expression of CA8 is listed in this chart, for $n=1803$ breast cancer patients in the lower survival quartile.