The brain-specific angiogenesis inhibitor 1-associated protein, BAIAP2, 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 the brain-specific angiogenesis inhibitor 1-associated protein, encoded by BAIAP2, 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. BAIAP2 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of BAIAP2 in primary tumors was significantly correlated with patient overall survival. Modulation of BAIAP2 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, the brain-specific angiogenesis inhibitor 1-associated protein, BAIAP2, 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 the brain-specific angiogenesis inhibitor 1-associated protein, BAIAP2, 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 BAIAP2 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using an unpaired two-tailed t-test with Welch's correction; PRISM 9.0 was utilized for statistical analyses. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of BAIAP2 mRNA expression levels with overall survival in $n=1402$ 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.

BAIAP2 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

1
2 brain-specific angiogenesis inhibitor 1-associated protein, encoded by BAIAP2, as a differentially
3 expressed gene in the breast metastatic tissues of humans with breast cancer (Table 1). When
4 sorting each of the genes expressed in brain metastases based on significance of difference as
5 compared to primary tumors of the breast in patients with breast cancer, BAIAP2 ranked 20 out
6 of 17418 total transcripts (Chart 1), equating to 99.9% differential expression. Differential
7 expression of BAIAP2 in the brain metastases of patients with metastatic breast cancer was
8 statistically significant (Chart 1; $p=2.05E-05$).

9
10 Differential gene expression analyses that utilize primary and metastatic tumor tissues to
11 discover genes associated with metastasis can be challenging due to the relatively heterogenous
12 cellular composition and constitution of the tumor as compared to normal, untransformed
13 (benign) tissues. Thus, to attempt to validate differential transcriptome-wide differential
14 expression of BAIAP2 in brain metastases in human metastatic breast cancer, we queried a
15 second microarray dataset⁵, here comparing normal breast tissues rather than primary tumors of
16 the breast to brain metastases. Again, we identified BAIAP2 as a differentially expressed gene in
17 the brain metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the
18 genes expressed in brain metastases based on significance of difference as compared to normal
19 breast tissues, BAIAP2 ranked 2403 out of 41093 total transcripts (Chart 2), equating to 94.2%
20 differential expression. Differential expression of BAIAP2 in the brain metastases of patients
21 with metastatic breast cancer was statistically significant (Chart 2; $p=8.98E-06$). Thus,
22 differential expression of BAIAP2, transcriptome-wide, in the brain metastases of women with
23 metastatic breast cancer was conserved across two independent microarray datasets, both when
24 compared to benign and transformed breast tissues.

25 **BAIAP2 is expressed at higher levels in the brain metastases of patients with metastatic** 26 **breast cancer.**

27 We obtained exact mRNA expression levels for BAIAP2, in primary tumors of the breast
28 and in brain metastasis of patients with brain metastatic breast cancer to determine direction and
statistical significance of change in BAIAP2 expression in brain metastatic tissues. BAIAP2 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;
 $p=0.0003$).

29 **BAIAP2 expression is significantly correlated with survival outcomes in human breast** 30 **cancer.**

31 We performed Kaplan-Meier survival analysis⁸ in 1402 breast cancer patients in total, to
32 evaluate whether BAIAP2 tumor expression was correlated with survival outcomes in breast
33 cancer. We observed a statistically significant correlation between primary tumor expression of
34 BAIAP2 and overall survival in patients with breast cancer, for patients in the upper survival
35 quartile (Figure 2). Patients whose primary tumors expressed low levels of BAIAP2 possessed
36 median OS of 115 months, while patients whose tumors expressed high levels of BAIAP2
37 possessed median OS of 62.07 months. This difference in OS based on BAIAP2 tumor
38 expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank
 p -value: 0.00093; hazard ratio: 1.48 (1.17-1.86)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the brain-specific angiogenesis inhibitor 1-associated protein, encoded by BAIAP2, 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 BAIAP2 in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between BAIAP2 expression and patient survival outcomes, as overall survival was significantly higher in patients whose primary tumors expressed lower levels of BAIAP2 as compared to patients whose primary tumors expressed higher levels of BAIAP2.

Discussion

We provided evidence here that the brain-specific angiogenesis inhibitor 1-associated protein, encoded by BAIAP2, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that BAIAP2 mRNA is present at significantly increased quantities in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor BAIAP2 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of BAIAP2 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of BAIAP2 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. Further, blinded identification of brain-specific differential gene expression in breast cancer cells that have metastasized to the brain demonstrates the power of our unbiased, systems-level discovery approach.

References

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Rank: 20
Probe ID: 1970
p-value: 2.05E-05
t: -5.98
B: 2.961804
Gene: BAIAP2
Gene name: the brain-specific angiogenesis inhibitor 1 (BAI1)-associated protein

Chart 1: BAIAP2 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: 2403
probe ID: A_23_P61810
p-value: 8.98E-06
t: -4.9813922
B: 3.26848
Gene: BAIAP2
Gene name: the brain-specific angiogenesis inhibitor 1-associated protein

Chart 2: BAIAP2 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: BAIAP2 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 BAIAP2 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 BAIAP2 expression between primary tumors of the breast and brain metastases is listed above as a *p*-value.

Figure 2: Significant correlation between BAIAP2 primary tumor expression and overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total patients stratified into two groups, based on low or high expression of BAIAP2 in patient primary tumors. 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 BAIAP2 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 BAIAP2 expression: 115 months
High BAIAP2 expression: 62.07 months

Chart 3: Median overall survival is inferior in patients with high primary tumor expression of BAIAP2, in the upper survival quartile.

The median OS (overall survival) of patients with low primary tumor expression of BAIAP2 and high primary tumor expression of BAIAP2 is listed in this chart, for $n=1402$ breast cancer patients, in the upper survival quartile.