PAPSS1 is differentially expressed 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 3'-phosphoadenosine 5'-phosphosulfate synthase 1, encoded by PAPSS1, 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. PAPSS1 mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of PAPSS1 in primary tumors was significantly correlated with patient overall survival. Modulation of PAPSS1 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, 3'-phosphoadenosine 5'-phosphosulfate synthase 1, PAPSS1, 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 3'-phosphoadenosine 5'-phosphosulfate synthase 1, PAPSS1, 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 $n=4$ 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 PAPSS1 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 PAPSS1 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.

PAPSS1 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 3'-phosphoadenosine 5'-phosphosulfate synthase 1, encoded by PAPSS1, 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, PAPSS1 ranked 185 out of 17418 total transcripts (Chart 1), equating to 98.9% differential expression. Differential expression of PAPSS1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p= 4.84e-04$).

To attempt to validate transcriptome-wide differential expression of PAPSS1 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 PAPSS1 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, PAPSS1 ranked 3462 out of 41093 total transcripts (Chart 2), equating to 91.6% differential expression. Differential expression of PAPSS1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=6.43e-03$). Thus, differential expression of PAPSS1, transcriptome-wide, in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets. This suggests that PAPSS1 differential expression in human brain metastatic breast cancer is a *bona fide* biological process.

PAPSS1 is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for PAPSS1, 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 PAPSS1 expression in brain metastatic tissues. We observed significantly lower expression of PAPSS1 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: PAPSS1 was expressed at 0.93 ± 0.49 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -0.51 ± 0.83 AU in brain metastatic tissues. The difference in PAPSS1 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.001079$).

PAPSS1 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=1402$ breast cancer patients in total, to evaluate whether PAPSS1 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of PAPSS1 and overall survival (OS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of PAPSS1 possessed median OS of 81 months, while patients whose tumors expressed high levels of PAPSS1 possessed median OS of 118.03 months. This difference in OS based on PAPSS1 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.04; hazard ratio: 0.8 (0.65-0.99)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified 3'-phosphoadenosine 5'-phosphosulfate synthase 1, encoded by PAPSS1, 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; we observed significantly decreased expression of PAPSS1 in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between PAPSS1 expression and patient survival outcomes, as overall survival was significantly greater in patients whose primary tumors expressed higher levels of PAPSS1 as compared to patients whose primary tumors expressed lower levels of PAPSS1.

Discussion

We provided evidence here that 3'-phosphoadenosine 5'-phosphosulfate synthase 1, encoded by PAPSS1, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that PAPSS1 mRNA is present at significantly decreased quantities in brain

1 metastatic tissues as compared to primary tumors of the breast, and that primary tumor PAPSS1 expression
2 is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects
3 of genetic depletion of PAPSS1 in mouse models of metastatic breast cancer on metastasis to the central
4 nervous system is merited. Modulation of PAPSS1 expression may be relevant to the processes by which
5 breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes,
6 evade immune clearance, breach the blood-brain barrier and colonize the brain.
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Rank: 185
Probe ID: 12613
p-value: 4.84e-04
t: 4.38
B: 0.076556
Gene: PAPSS1
Gene name: 3'-phosphoadenosine 5'-phosphosulfate synthase 1

Chart 1: PAPSS1 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: 3462
probe ID: 8102214
p-value: 6.43e-03
t: 3.0410864
B: -2.594248
Gene: PAPSS1
Gene name: 3'-phosphoadenosine 5'-phosphosulfate synthase 1

Chart 2: PAPSS1 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: PAPSS1 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 PAPSS1 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 PAPSS1 expression between primary tumors of the breast and brain metastases, a *p*-value, is *p*=0.001079.

Figure 2: Significant correlation between PAPSS1 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 PAPSS1 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 PAPSS1 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 PAPSS1 expression: 81 months
High PAPSS1 expression: 118.093 months

Chart 3: Median overall survival is inferior in patients with low primary tumor expression of PAPSS1.

The median OS (overall survival) of patients with low primary tumor expression of PAPSS1 and high primary tumor expression of PAPSS1 is listed in this chart, for $n=1402$ breast cancer patients.