PSENEN 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 presenilin enhancer, gamma-secretase subunit, encoded by PSENEN, 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 breast⁴. PSENEN mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of PSENEN in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of PSENEN 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, presenilin enhancer, gamma-secretase subunit, PSENEN, 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 presenilin enhancer, gamma-secretase subunit, PSENEN, 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 metastatic 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 PSENEN 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 PSENEN mRNA expression levels with recurrence-free survival in $n=3951$ breast cancer patients, and with distant metastasis-free survival in $n=1803$ 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.

PSENEN 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

presenilin enhancer, gamma-secretase subunit, encoded by PSENEN, 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, PSENEN ranked 91 out of 17418 total transcripts (Chart 1), equating to 99.5% differential expression. Differential expression of PSENEN in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.82E-04$).

To attempt to validate transcriptome-wide differential expression of PSENEN 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 PSENEN 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 primary tumors of the breast, PSENEN ranked 1653 out of 33297 total transcripts (Chart 2), equating to 95.04% differential expression. Differential expression of PSENEN in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=9.13E-04$). Thus, differential expression of PSENEN, transcriptome-wide, in brain metastatic tissues of patients with metastatic breast cancer could be observed across two independent microarray datasets.

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

We obtained exact mRNA expression levels for PSENEN, 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 PSENEN expression in brain metastatic tissues. PSENEN 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; $p=0.000228$).

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

We performed Kaplan-Meier survival analysis⁷ in 3951 breast cancer patients in total, to evaluate whether PSENEN tumor expression was correlated with survival outcomes in human breast cancer. We observed a statistically significant correlation between primary tumor expression of PSENEN and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of PSENEN possessed median RFS of 55 months, while patients whose tumors expressed high levels of PSENEN possessed median RFS of 45.93 months. This difference in RFS based on PSENEN tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.0016; hazard ratio: 1.19 (1.07-1.33)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified presenilin enhancer, gamma-secretase subunit, encoded by PSENEN, 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 breasts; we observed significantly increased expression of PSENEN in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between PSENEN expression and patient survival outcomes, as recurrence-free survival was significantly greater in patients whose primary tumors expressed lower levels of PSENEN as compared to patients whose primary tumors expressed higher levels of PSENEN.

Discussion

We provided evidence here that the presenilin enhancer, gamma-secretase subunit, encoded by PSENEN, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that PSENEN mRNA is present at significantly increased quantities in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor PSENEN expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of PSENEN in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. We recently described transcriptome-wide differential expression of the delta-like non-canonical Notch ligand 1, DLK1, in the brain and lymph node metastases of patients with metastatic breast cancer⁸, together suggesting that perturbations in the Notch signaling pathway may be important for metastatic progression of breast cancer in humans. Modulation of PSENEN 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.

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Rank: 91
Probe ID: 11904
P-value: 1.82E-04
t: -4.86
B: 0.978664
Gene: PSENEN
Gene name: presenilin enhancer, gamma-secretase subunit

Chart 1: PSENEN 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: 1653
probe ID: 8027996
p-value: 9.13E-04
t: -3.8859797
B: -0.747283
Gene: PSENEN
Gene name: presenilin enhancer, gamma-secretase subunit

Chart 2: PSENEN 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.

PSENEN

Figure 1: PSENEN 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 PSENEN 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 PSENEN expression between primary tumors and brain metastasis, a p -value, is $p=0.000228$.

Figure 2: Significant correlation between PSENEN primary tumor expression and recurrence-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=3951$ total patients stratified into two groups, based on low or high expression of PSENEN in patient primary tumors. The log rank p -value denoting statistical significance of difference in recurrence-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 PSENEN 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 PSENEN expression: 55 months
High PSENEN expression: 45.93 months

Chart 3: Median recurrence-free survival is inferior in patients with high primary tumor expression of PSENEN.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of PSENEN and high primary tumor expression of PSENEN is listed in this chart, for $n=3951$ breast cancer patients.